PB83-135970

Health Assessment Document for Trichloroethylene

(U.S.) Environmental Protection Agency
Research Triangle Park, NC

Jan 82

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA-600/8-82-006
March 1982

PB83-135970

Research and Development

Health Assessment Document for Trichloroethylene

DRAFT

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/8-82-006

2.

3. RECIPIENT'S ACCESSION NO.
PB83 135970

4. TITLE AND SUBTITLE
Health Assessment Document for Trichloroethylene

5. REPORT DATE
January 1982

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
I.W.F. Davidson, Wake Forest Univ.; Mark M. Greenberg;
Jean C. Parker

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Criteria and Assessment Office
MD-52
Research Triangle Park, NC 27711

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Office of Research and Development
Office of Health and Environmental Assessment (RD-689)
401 M Street, SW, Washington, DC 20460

13. TYPE OF REPORT AND PERIOD COVERED
Draft

14. SPONSORING AGENCY CODE
EPA/600/00

15. SUPPLEMENTARY NOTES
This document supercedes PB 82-183476

16. ABSTRACT

Exposure to trichloroethylene is associated with neurological disorders, cardiovascular effects and damage to the liver and kidney. Liver and kidney damage have been attributed to chronic high exposure (>1,000 ppm). It is highly likely that the direct myocardial depressent effect, which is a serious health hazard for those with compromised or reduced cardiac reserve, occurs at lower exposure concentrations than has been previously thought.

Although animal teratology studies performed to date are inconclusive, trichloroehtylene could be teratogenic since: (1) it is distributed in the fetus, (2) biotransformation into reactive metabolites occurs in fetal liver, and (3) trichloroethylene has high lipid solubility.

There is evidence that epoxide-stabilized trichloroethylene has carcinogenic activity. An increased incidence of hepatocellular carcinomas in exposed B6C3F1 mice was observed. Technical grade trichloroethylene produced positive responses in mutagenicity assay systems with bacteria and yeast. Analytic grade trichloroethylene caused somatic mutations in the mouse spot test and induced cell transformation *in vitro* in a Fischer F1706 rat embryo cell line. In addition, weak mutagenic responses were produced in bacteria and yeast strains. Based on the weight of evidence, it is concluded that trichloroethylene is a weak mutagen.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
Distribute to Public

19. SECURITY CLASS *(This Report)*
Unclassified

20. SECURITY CLASS *(This page)*
Unclassified

21. NO. OF PAGES
357

22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

i

EPA-600/8-82-006
Review Draft

DRAFT
Do not cite or quote

HEALTH ASSESSMENT DOCUMENT FOR TRICHLOROETHYLENE

Notice

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Environmental Criteria and Assessment Office
Research Triangle Park, North Carolina 27711

Project Coordinator: Dr. Jean C. Parker

i.a

DISCLAIMER

This report is an internal draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assessment to serve as a "source document" for Agency-wide use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards, however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects. This assessment will help insure consistency in the Agency's consideration of the relevant scientific health data associated with trichloroethylene.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

TABLE OF CONTENTS

Page

LIST OF TABLES........ vii
LIST OF FIGURES........ viii

1. SUMMARY AND CONCLUSIONS........ 1-1

2. INTRODUCTION........ 2-1

3. BACKGROUND INFORMATION........ 3-1
  3.1. PHYSICAL AND CHEMICAL PROPERTIES........ 3-1
  3.2 ENVIRONMENTAL FATE AND TRANSPORT........ 3-4
    3.2.1 Production........ 3-4
    3.2.2 Use........ 3-6
    3.2.3 Emissions........ 3-7
    3.2.4 Persistence........ 3-8
    3.2.5 Degradation........ 3-11
  3.3 LEVELS OF EXPOSURE........ 3-12
    3.3.1 Analytical Methodology........ 3-12
      3.3.1.1 Gas Chromatography with Electron Capture Detection........ 3-12
      3.3.1.2 Gas Chromatography-Mass Spectrometry........ 3-15
    3.3.2 Calibration........ 3-16
    3.3.3 Sampling of Ambient Air and Water........ 3-17
      3.3.3.1 Air-Mixing Ratios........ 3-20
      3.3.3.2 Water........ 3-20
      3.3.3.3 Soil and Sediment........ 3-28
  3.4 ECOLOGICAL EFFECTS........ 3-28
  3.5 CRITERIA, STANDARDS, AND REGULATIONS........ 3-30
  3.6 RELATIVE SOURCE CONTRIBUTIONS........ 3-31
  3.7 REFERENCES........ 3-33

4. MAMMALIAN DISPOSITION AND FATE........ 4-1
  4.1 INTRODUCTION........ 4-1
  4.2 ABSORPTION AND DISTRIBUTION........ 4-2
    4.2.1 Oral........ 4-2
    4.2.2 Dermal........ 4-4
    4.2.3 Pulmonary Absorption and Tissue Distribution........ 4-5
    4.2.4 Pharmacokinetics of TCI Disposition........ 4-7
  4.3 EXCRETION........ 4-8
    4.3.1 Pulmonary Elimination........ 4-8
    4.3.2 Urinary Metabolite Excretion........ 4-9
  4.4 MEASURES OF EXPOSURE AND BODY BURDEN........ 4-11
  4.5 BIOTRANSFORMATION........ 4-12
    4.5.1 Enzymatic Pathways of Biotransformation........ 4-16
    4.5.2 Trichloroethylene Expoxide Formation and Covalent Binding........ 4-16
    4.5.3 Metabolism of Chloral Hydrate........ 4-22
  4.6 SPECIES DIFFERENCES IN MAGNITUDE OF TCI METABOLISM 4-24
  4.7 TCI METABOLISM AND DRUG INTERACTIONS........ 4-27
  4.8 METABOLISM AND TOXICITY........ 4-30
  4.9 REFERENCES........ 4-32

Page

5. TOXICOLOGICAL EFFECTS IN MAN AND EXPERIMENTAL ANIMALS.. 5-1
  5.1 INTRODUCTION 5-1
  5.2 EFFECTS ON THE CENTRAL NERVOUS SYSTEM 5-1
    5.2.1 Human Studies: Acute Inhalation Exposure.. 5-1
    5.2.2 Human Studies: Chronic Exposure 5-4
    5.2.3 Experimental Human Studies 5-5
      5.2.3.1 Controlled Studies 5-5
      5.2.3.2 Epidemiological Studies 5-6
    5.2.4 Animal Studies 5-7
  5.3 EFFECTS ON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS 5-9
    5.3.1 Cardiovascular Effects 5-9
    5.3.2 Respiratory Effects 5-11
  5.4 HEPATIC AND RENAL TOXICITY 5-12
    5.4.1 Human Studies 5-12
    5.4.2 Animal Studies 5-14
  5.5 DERMAL EFFECTS 5-16
  5.6 MODIFICATION OF TOXICITY: SYNERGISM AND ANTAGONISM 5-16
  5.7 SUMMARY OF TOXIC EFFECTS 5-19
  5.8 REFERENCES 5-24

6. TERATOGENIC AND FETOTOXIC POTENTIAL 6-1
  6.1 ANIMAL STUDIES 6-3
    6.1.1 Avian Embryo System 6-3
    6.1.2 Mouse 6-5
    6.1.3 Rat 6-6
    6.1.4 Rabbit 6-11
  6.2 FETOTOXICITY IN HUMANS 6-13
  6.3 SUMMARY 6-15
  6.4 REFERENCES 6-17

7. CARCINOGENIC POTENTIAL 7-1
  7.1 MUTAGENICITY AND CELL TRANSFORMATION 7-5
  7.2 CELL TRANSFORMATION 7-6
  7.3 ANIMAL BIOASSAYS 7-6
    7.3.1 NCI (1976) 7-9
    7.3.2 MCA (1978) 7-11
    7.3.3 Maltoni (1979) 7-13
    7.3.4 Henschler (1980) 7-13
    7.3.5 Van Duuren (1979) 7-15
  7.4 EPIDEMIOLOGIC STUDIES 7-16
  7.5 PERSPECTIVE 7-18
  7.6 REFERENCES 7-21

8. SUMMARY OF ADVERSE HEALTH EFFECTS AND LOWEST OBSERVED EFFECT LEVELS 8-1
  8.1 SUMMARY OF TOXIC EFFECTS 8-1
    8.1.1 Animal Toxicity Studies Useful for Hazard Assessment 8-1

8.2 INHALATION EXPOSURE.................................. 8-2
8.2.1 Effects of Single or Acute Inhalation Exposure.................................. 8-3
8.2.2 Effects of Intermittent or Prolonged Inhalation Exposure.......................... 8-6
8.2.3 Estimated Threshold Effects Levels......... 8-9
8.3 ORAL EXPOSURE.................................. 8-10
8.3.1 Effects of Oral Exposure.................. 8-10
8.3.2 Estimated Threshold Effects Levels......... 8-13
8.4 DERMAL EXPOSURE.................................. 8-14
8.5 REFERENCES.................................. 8-15

9. COLLATED BIBLIOGRAPHY.................................. 9-1

APPENDIX A (CARCINOGEN ASSESSMENT OF TRICHLOROETHYLENE)..... A-1

LIST OF TABLES

Number Page

3-1 Physical Properties of Trichloroethylene................ 3-1
3-2 Stabilizers in Trichloroethylene Formulations.......... 3-3
3-3 Partition Coefficients of Trichloroethylene and Other Chlorinated Hydrocarbons......................... 3-4
3-4 TCI Production and Consumption......................... 3-5
3-5 U.S. Production Capacity for Trichloroethylene......... 3-5
3-6 Miscellaneous Uses of Trichloroethylene................ 3-7
3-7 Estimated Half-life of TCI in Surface Waters........... 3-10
3-8 Ambient Air Mixing Ratios of Trichloroethylene......... 3-21
3-9 Mean Levels of TCI in Surface Waters................... 3-25
3-10 Indications of Relative Exposure for Trichloroethylene. 3-32

4-1 Partition Coefficients of TCI for Various Body Tissues of Rat and Man................................. 4-3
4-2 Differences in Metabolism of TCI Urinary Excretion Metabolites (mg)....................................... 4-23

5-1 Toxic Dose Data.. 5-20
5-2 Occupational Industrial Hygiene Standards for Trichloroethylene...................................... 5-23

6-1 Summary of Animal Studies of Fetotoxic and Teratogenic Potential of TCI........................... 6-4
6-2 Experimental Design of Beliles et al. Study (1980) on Sprague-Dawley Rats................................ 6-8
6-3 Experimental Design of Dorfmueller et al. Study (1979) on Long-Evans Rats.................................... 6-9
6-4 Experimental Design of Beliles et al. Study (1980) on New Zealand White Rabbits.......................... 6-11

7-1 Chronology of Mutagenic Tests of TCI.................. 7-3
7-2 TCI Carcinogenicity Bioassays in Animals............... 7-7
7-3 Incidence of Hepatocarcinoma in B6C3F1 Mice Exposed to TCI (NCI)................................... 7-10
7-4 Incidence of Hepatocarcinoma in B6C3F1 Mice Exposed to TCI (MCA)... 7-12
7-5 Incidence of Malignant Lymphoma in Femal HAN:NMRI Mice Exposed to TCI.. 7-14

LIST OF FIGURES

Number		Page
4-1	Trichloroethylene epoxide formation and rearrangements....	4-14
4-2	Metabolic transformations of chloral hydrate...............	4-17
4-3	Relationship between environmental trichloroethylene concentration and urinary excretion of trichloroethylene metabolites in human urine.................................	4-26

The authors of this document are:

Dr. I. W. F. Davidson, Department of Physiology/Pharmacology, The Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina.

Mr. Mark M. Greenberg, Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina.

Dr. Jean C. Parker, Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina.

The following individuals reviewed an early draft of this document and submitted valuable comments:

Dr. Joseph Borzelleca
Department of Pharmacology
The Medical College of Virginia
Health Sciences Division
Virginia Commonwealth University
Richmond, Virginia 23298

Dr. Herbert Cornish
Dept. of Environmental and Industrial Health
University of Michigan
Ypsilanti, Michigan 48197

Dr. I. W. F. Davidson
Dept. of Physiology/Pharmacology
The Bowman Gray School of Medicine
300 S. Hawthorne Road
Winston-Salem, North Carolina 27103

Dr. Lawrence Fishbein
National Center for Toxicological Research
Jefferson, Arkansas 72079

Dr. John G. Keller
P.O. Box 12763
Research Triangle Park, North Carolina 27709

Dr. Marvin Legator
Professor and Director
Dept. of Preventive Medicine and
  Community Health
Div. of Environmental Toxicology and
  Epidemiology
University of Texas Medical Branch
Galveston, Texas 77550

Dr. Benjamin Van Duuren
Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016

The following persons attended a review workshop to discuss EPA draft documents on organics which included an early draft of this document.

Dr. Mildred Christian
Argus Laboratories, Inc.
Perkasie, Pennsylvania 18944

Dr. Herbert Cornish
Dept. of Environmental and Industrial Health
University of Michigan
Ypsilanti, Michigan 48197

Dr. I. W. F. Davidson
Dept. of Physiology/Pharmacology
The Bowman Gray School of Medicine
300 S. Hawthorne Road
Winston-Salem, North Carolina 27103

Dr. John Egle
Dept. of Pharmacology
Virginia Commonwealth University
Richmond, Virginia 23298

Dr. Thomas Haley
National Center for Toxicological Research
Jefferson, Arkansas 72079

Dr. Rudolph J. Jaeger
Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016

Dr. John G. Keller
P.O. Box 12763
Research Triangle Park, North Carolina 27709

Dr. Norman Trieff
Dept. of Preventive Medicine
University of Texas Medical Branch
Galveston, Texas 77550

Dr. Benjamin Van Duuren
Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016

Dr. James Withey
Food Directorate
Bureau of Food Chem.
Tunney's Pasture
Ottawa, Canada

Participating Members of the Carcinogen Assessment Group

Roy C. Albert, M.D., Chairman
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.
Margaret M.L. Chu, Ph.D.
Herman J. Gibb, B.S., M.P.H.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert McGaughy, Ph.D.
Dharm V. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

Participating Members of the Reproduction Effects Assessment Group

John R. Fowle III, Ph.D.

Members of the Agency Work Group on Solvents

Elizabeth L. Anderson	Office of Health and Environmental Assessment
Charles H. Ris	Office of Health and Environmental Assessment
Jean C. Parker	Office of Health and Environmental Assessment
Mark M. Greenberg	Office of Health and Environmental Assessment
Cynthia Sonich	Office of Health and Environmental Assessment
Steve Lutkenhoff	Office of Health and Environmental Assessment
James A. Stewart	Office of Health and Environmental Assessment
Paul Price	Office of Toxic Substances
William Lappenbush	Office of Toxic Substances
Hugh Spitzer	Office of Drinking Water
David R. Patrick	Consumer Product Safety Commission
Lois Jacob	Office of Air Quality Planning and Standards
Arnold Edelman	Office of General Enforcement
Josephine Brecher	Office of Toxic Integration
Mike Ruggiero	Office of Water Regulations and Standards
Jan Jablonski	Office of Water Regulations and Standards
Charles Delos	Office of Solid Waste
Richard Johnson	Office of Water Regulations and Standards
Priscilla Holtzclaw	Office of Pesticide Programs
	Office of Emergency and Remedial Response

1. SUMMARY AND CONCLUSIONS

Trichloroethylene [1,1,2-trichloroethylene (TCI)] is a solvent widely used in the industrial degreasing of metals. It has no known natural sources. Current United States production is estimated at about 130,000 metric tons per year. Eighty to 95 percent of the trichloroethylene used in the United States evaporates to the atmosphere. In addition to the workplace, trichloroethylene is found in a variety of urban and nonurban areas of the United States and other regions of the world. It has been measured in ambient air and water. An average ambient air concentration of about 1 part per billion (ppb) would be expected for some large urban centers. Concentrations as high as 32 ppb have been measured in urban centers in the United States, and 47 ppb has been measured in urban Tokyo.

Ambient air concentrations of trichloroethylene are greatly influenced by the rate and geographic distribution of emissions and the rate of decomposition and transposition in the atmosphere. The average mixing ratio in the troposphere of the northern hemisphere is 11 to 17 ppt. Reaction with hydroxyl radicals is the principal mechanism by which trichloroethylene is scavenged from the atmosphere. The photoreactive decomposition of trichloroethylene in the atmosphere results in the production of phosgene and dichloroacetyl chloride, both of which are toxic.

Trichloroethylene has been detected in both natural and municipal waters in the United States. Up to 403 ppb have been measured in surface and subsurface waters, and in finished drinking water concentrations have ranged from 1 to 32 ppb. There is no direct evidence of bioaccumulation of trichloroethylene in the food chain, although various natural products (milk, cheese, etc.) have been found to contain trichloroethylene. Few studies have been made of the ecological consequences of trichloroethylene in the environment.

The pharmacokinetics and metabolism of trichloroethylene have been studied in man as well as in animals. As with other related halogenated aliphatic solvents, the principal route of exposure to trichloroethylene is by inhalation of vapor in air followed by lung absorption. Also trichloroethylene is very well absorbed into the body after oral ingestion. Both exposure routes are important because trichloroethylene contaminates air, water and food. Ingested trichloroethylene enters the portal system first perfusing the liver: thus, at low concentrations only a small proportion of trichloroethylene would enter the general circulation because uptake and metabolism by the liver are expected to be nearly complete. In contrast, inhaled trichloroethylene directly enters the systemic circulation and is widely distributed in the body.

Because of its lipophilic nature, trichloroethylene readily crosses lipid-containing cell membranes and concentrates selectively in adipose tissue, where it has a residence half-time of 3.4 hr. in humans. Of special toxicological importance is the fact that trichloroethylene enters the brain, the milk of nursing mothers and, the fetus. Evidence exists that trichloroethylene accumulates in the body with chronic exposure to low ambient concentrations: it has been detected in the breath of healthy humans (not occupationally exposed) and in post mortem samples of human adipose tissue.

The amount of trichloroethylene absorbed after oral ingestion is virtually complete; with air exposure, the amount of trichloroethylene absorbed increases in proportion to its concentration in inspired air, the respiratory rate, and duration of exposure. Trichloroethylene is eliminated by two major processes, pulmonary excretion of unchanged trichloroethylene and liver metabolism to urinary metabolites. At air concentrations of 500 ppm or less, humans metabolize 60 to 90 percent of absorbed trichloroethylene. Studies have not been made of metabolism after oral exposure in man; however, an even higher proportion of an absorbed amount of trichloroethylene is expected to be metabolized following exposure by this route. The principal identified urinary metabolites of trichloroethylene are trichloroethanol (TCE) and its glucuronide, and trichloroacetic acid (TCA). The evidence is strong that, in the liver, trichloroethylene is first metabolized to a reactive epoxide (trichloroethylene oxide) by a microsomal P_{450} system. This reactive epoxide covalently binds extensively to cellular macromolecules. The epoxide is further converted to chloral hydrate and thence to TCE and TCA. Metabolism of trichloroethylene is enhanced by drugs and xenobiotics such as barbiturates, oral antidiabetic agents, and PCBs, which induce the hepatic microsomal P_{450} metabolizing system. Heightened toxicity results from clinical drug interactions known to occur with ethanol, barbiturates, disulfiram and Warfarin.

Exposure to trichloroethylene is associated with neurological disorders, cardiovascular effects, and morphological and functional damage to the liver and kidney. The intensity of these toxic effects resulting from inhalation exposure depends greatly on vapor concentration in the ambient air, duration of exposure, and individual susceptibility. Acute exposure to high concentrations (>1000 ppm) of trichloroethylene narcotizes the central nervous system (CNS), progressively depressing all functions of the brain from cortex to medulla. Short exposures (few minutes) result in headache, nausea, and incoordination; longer exposures cause CNS depression and unconsciousness, increased respiratory rate with decreased ventilation amplitude, increased cardiac output, and cardiac arrhythmias. Sudden death following exposure to trichloroethylene has been attributed to a direct myocardial action sensitizing the heart to circulating catecholamines which results in ventricular fibrillation and cardiac arrest.

The liver and the kidneys are major target organs of trichloroethylene toxicity. Fatty liver, liver enlargement, abnormal liver function, as well as kidney damage--especially to the renal tubules--have been attributed to chronic high exposure (> 1000 ppm) to trichloroethylene.

There is less agreement about the human toxicity of trichloroethylene from lower-level (50-500 ppm), long-term exposures. Reports of the toxicological consequences of industrial exposures are often sketchy, and there are few well-controlled epidemiological studies. Difficulties in delineating the toxic effects of trichloroethylene are further compounded by chemical impurities and toxic decomposition products of trichloroethylene. However, experimental studies in human volunteers provide some information about the relationship between chronic low-level exposure to trichloroethylene and toxic effects. Signs and symptoms of toxicity include dizziness, headache, fatigue, nausea, fainting spells, and other subjective responses that suggest a CNS origin. Dermal and eye irritation and intolerance to alcohol are among the better defined manifestations of exposure to trichloroethylene. Behavioral and psychological effects, particularly as they affect manual and mental performance, have been reported at levels of 100 ppm (current TWA-TLV) in some, but not all, experimental and epidemiological studies. It is highly likely that the direct myocardial depressant effect, which is a serious health hazard for those with compromised or reduced cardiac reserve occurs at lower exposure concentrations than has been previously thought.

The potential for trichloroethylene to cause adverse teratogenic, mutagenic, and carcinogenic effects in humans at low levels of exposure is an important consideration; however, no well-constructed epidemiological studies have been carried out that evaluate effects of chronic exposure to low concentrations on human health. Information from experimental animal studies suggests that trichloroethylene could be teratogenic since: a) it has been shown to distribute in the fetus b) hepatic biotransformation into cellular reactive metabolites has been shown to occur in fetal liver, and c) high lipid solubility of trichloroethylene in cellular membranes occurs with storage in adipose tissue. However, animal teratology studies performed to date are inconclusive and do not provide adequate evidence to indicate that trichloroethylene may have teratogenic potential in humans. Reports of teratogenic hazard in humans is limited to scattered observations in the literature and newspaper reports of trichloroethylene exposure associated with miscarriages, a stillbirth, and neonatal respiratory and neuromuscular problems. However, these anecdotal reports are insufficient for establishing a scientific basis for this association. Case reports of sacral agenesis have been reported in infants whose mothers were exposed to organic solvents during pregnancy; however, a causal relationship to trichloroethylene exposure alone was not established. The available data therefore is inconclusive and further testing would be required to define the potential for teratogenesis of trichloroethylene in humans.

There is evidence that technical grade trichloroethylene (epoxide-stabilized) has carcinogenic activity, based on the increased incidence of hepatocellular carcinomas in exposed B6C3F1 mice, the positive mutagenic responses in bacteria and yeast, and the positive mutagenic response of bacteria to chloral hydrate. Applying the International Agency for Research on Cancer (IARC) criteria for animal studies, this level of evidence would be regarded as limited and not sufficient to provide a firm conclusion on its carcinogenic potential in humans.

In addition, evidence that purified grade trichloroethylene has carcinogenic activity consists of the increased lymphoma incidence in Han:NMRI mice; the positive mutagenic responses in bacteria, yeast, and the mouse spot test in the presence of purified (analytical) grade trichloroethylene and in bacteria exposed to chloral hydrate; and the positive response in the in vitro cell transformation study in Fischer F1706 rat embryo cells with purified (analytical) grade trichloroethylene. Applying the IARC criteria for animals, this level of evidence would be regarded as limited and not sufficient to provide a firm conclusion on its carcinogenic potential in humans. Furthermore, the relatively high spontaneous incidence of lymphoma and the possibility of an indirect mechanism of response in the Han:NMRI mice make the evidence of carcinogenicity for purified trichloroethylene weaker than for the technical material.

Technical grade trichloroethylene, administered at maximally tolerated and one-half maximally tolerated dosages by gavage for 78 weeks, was shown to induce a statistically significant increase in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice; metastases were also found in males in both treatment groups. In a second lifetime study, in which mice were exposed to technical grade trichloroethylene vapor by inhalation for 24 months, a statistically significant increase in the incidence of hepatocellular carcinomas in male B6C3F1 mice was observed; however, this study is weakened by wide variability in the trichloroethylene exposure concentrations and the lack of concurrent matched controls. Two chronic rat studies with technical grade trichloroethylene, one with Osborne-Mendel rats by gavage and the other with Charles River rats by inhalation, showed no carcinogenic response. In a study of female Han:NMRI mice exposed to purified grade trichloroethylene vapor for 18 months, a statistically significant increase in the incidence of malignant lymphomas was found; trichloroethylene was not observed to be carcinogenic in Han:Wist rats, Syrian hamsters, and male Han:NMRI mice when dosed similarly. The B6C3F1 mice in the first two studies were treated with technical grade trichloroethylene contaminated with the epoxides epichlorohydrin and 1,2-epoxybutane, whereas the Han:NMRI mice, the Han:Wist rats, and the hamsters were exposed to purified trichloroethylene containing no detectable epoxides.

It is possible that the induction of malignant lymphomas in female Han:NMRI mice by purified grade trichloroethylene could have been due to an immunosuppressive effect of trichloroethylene; however, since little is known about the dose-response relationship for immunosuppressive agents, the significance of trichloroethylene exposure at doses considerably below those to which the Han:NMRI mice were exposed on immunosuppression is not clear.

Other chronic animal studies have been reported which indicate that trichloroethylene of undetermined purity, administered by gavage or inhalation, was not carcinogenic; however, duration of treatment was either less than the lifetime of the animals or, in one study, not specified.

In lifetime studies of ICR/Ha Swiss mice treated with purified grade trichloroethylene at doses below that maximally tolerated, no carcinogenic response was observed via dermal application in females, or with once-weekly administration by gavage or subcutaneous injection. Neither trichloroethylene nor trichloroethylene epoxide was found to have initiating activity in a lifetime initiation-promotion experiment in female ICR/Ha Swiss mice treated with the same initiating dose as that used in the repeated dosing studies. Trichloroethylene epoxide applied topically and subcutaneously in female ICR/Ha Swiss mice induced no carcinogenic response at the site of application, according to prepublication data; no indication of the maximum tolerated dose in these experiments is available.

The carcinogenicity of trichloroethylene, devoid of detectable epoxides, is being evaluated by the National Toxicology Program (NTP) with gavage administration, and by C. Maltoni and Associates via inhalation exposure in ongoing studies in various strains of mice and rats. The results of these studies should provide more definitive information about the carcinogenicity of purified trichloroethylene.

A number of studies have been conducted to assess the mutagenic potential of trichloroethylene, including primary DNA damage, chromosomal aberrations, gene mutation, alterations in cell growth and mitosis, and ability of the compound to reach the gonads of an intact mammal. The results of these studies have been conflicting, but because of trichloroethylene's high volatility and low solubility it has been difficult to adequately test this material. Several investigators reporting negative results possibly failed to take these properties into account and the concern is raised that the target cells were not adequately exposed. Furthermore, trichloroethylene is unstable and the chemical substances which have been employed to stabilize it have often been potent mutagens. Therefore, positive results reported in studies using technical grade samples known to contain mutagenic stabilizers or reports of studies which do not contain information concerning the purity of the tested material should be viewed with caution. In such cases it is not possible to ascribe any mutagenic response to trichloroethylene.

Concerning the testing of technical grade trichloroethylene for mutagenicity, positive responses were observed with the base pair substitution-detecting Salmonella typhimurium strains TA 100 and TA 1535 and the Saccharomyces cerevisiae XV 185-14C haploid strain both with and without metabolic activation. Similarly, it was reported that human exposure to technical grade trichloroethylene results in an increase in hypodiploid cells. It has also been reported that technical grade trichloroethylene causes somatic cell mutations in Tradescantia and alters cell growth and mitosis in Chinese hamster fibroblast cells and Euglena. Negative results have been reported after testing technical grade trichloroethylene in Salmonella typhimurium strain TA 100 with metabolic activation, in Schizo saccharomyces pombe host-mediated assays in mice, and in a Drosophila melanogaster sex-linked recessive lethal test. Purified (analytical) grade trichloroethylene produced weak (less than twofold) mutagenic responses in the presence of metabolic activation in three tests conducted using S. typhimurium strain TA 100, three tests conducted using S. cerevisiae strains D4 and D7, and one test using Escherichia coli K12 strain. Analytic grade trichloroethylene was also demonstrated to cause somatic mutations in the mouse spot test and to induce cell transformation in vitro in a Fischer F1706 rat embryo cell line. Negative responses were reported in two tests conducted with Salmonella strains, one dominant lethal study employing mice, and one study of the ability of trichloroethylene to cause SCEs in Chinese hamster ovary cells. Evidence is available to indicate that trichloroethylene is metabolically converted to a reactive epoxide intermediate, and epoxides as a group are considered to be potentially carcinogenic. Chloral hydrate, a metabolite of trichloroethylene, was shown to be weakly mutagenic (less than twofold increase) in a S. typhimurium TA 100/rat liver microsome assay. Based on the weight of evidence available at this time, it is concluded that trichloroethylene is a weak mutagen.

Research Needs: The toxicity of long-term, low-level trichloroethylene exposure is poorly defined in both human and experimental animals. Well-controlled epidemiologic studies that assess not only cancer risk but also other potential adverse health effects (teratogenic, mutagenic, CNS, cardiac) are lacking. Well-designed, double-blind studies of potential CNS effects in human subjects would resolve the question of whether manual and mental performance is impaired at industrial levels of exposure to trichloroethylene. It is likely that the direct myocardial depressant effect that has been seen following acute exposure to high concentrations of trichloroethylene also occurs to some degree at much lower concentrations. Studies to measure myocardial depression at low chronic exposure might be carried out to assess this hazard. Clarification of the significance of the observed teratogenic effects is needed. In particular, perinatal target organ toxicity studies and additional post-natal studies in laboratory animals should be helpful. Studies are currently in progress (NCI; Maltoni) to determine whether pure trichloroethylene is carcinogenic in mice and rats; these studies may provide some information on the chronic oral and inhalation toxicity of trichloroethylene. Little information on the ecological effects of trichloroethylene is available.

ADDENDUM

The Carcinogen Assessment Group recently examined the Preliminary Review of the National Toxicology Program's (NTP) Carcinogensis Bioassay of Trichloroethylene, dated October 23, 1981 (NIH 82-1799).* This bioassay administered pure "Hi-Tri" trichloroethylene (free of epichlorohydrin stabilizers) in corn oil by gavage to groups of 50 male and female Fischer 344 rats at doses of 500 or 1000 mg/kg and two groups of 50 male and female B6C3F1 mice at doses of 1000 mg/kg, dosed five times per week for up to 103 weeks. This preliminary draft reported statistically significant incidences of renal carcinomas or adenocarcinomas in male F344 rats, statistically significant incidences of hepatocellular carcinomas in both male and female B6C3F1 mice, and the unusual occurrence of squamous-cell papillomas and a squamous-cell carcinoma of the stomach in the treated female mice. It should be noted that this preliminary report supports the carcinogenicity of pure trichloroethylene and that carcinogenicity occurred in two species, which would cause it to be categorized as having sufficient evidence of carcinogenicity by the IARC criteria. This study cannot be fully evaluated until it is released in its final form, which is expected to be sometime in June 1982.

---

*The following disclaimer was attached to this NTP report: "This is not a final report. Until this DRAFT is reviewed and approved by the Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors, this DRAFT does not represent the official position of the National Toxicology Program."

2. INTRODUCTION

Trichloroethylene is one of the most widely used industrial chemicals in the United States. Following a decline in production and use of trichloroethylene in favor of other chlorinated hydrocarbon solvents, the manufacture and consumption have fluctuated in recent years, and no clear trend has been established. The 1979 production was 145 thousand metric tons,[1] 80 percent of which was used in vapor degreasing of fabricated metal parts; the remainder was used in a miscellany of other industrial solvent applications and in consumer products. Nearly all of each year's production of trichloroethylene represents replacement of evaporative loss to the environment. In the past, primary concern for the potential adverse health effects of trichloroethylene was directed to the health of exposed industrial workers. Recently, this concern has broadened due to the discovery and realization that the release of trichloroethylene into the atmosphere has also contaminated raw water sources, drinking water, soil and, possibly, the food chain. The implication of such widespread exposure is not only that the size of the population exposed is greatly increased but also that the population exposure includes substantial subpopulations who may be particularly susceptible to the adverse health effects of trichloroethylene, i.e., pregnant women, infants and children, the elderly, and persons taking certain medications with which trichloroethylene may produce adverse clinical interactions.

This document provides a discussion of the health hazards of trichloroethylene and indicates the concentration or dose levels at which adverse effects might be expected to occur.

---

[1] Handbook of Economics, 1980.

Both the older and current scientific literature has been comprehensively reviewed and evaluated. Throughout the document the abbreviation TCI has been used for trichloroethylene; TCI is the abbreviation used for trichloroethylene by many prominent investigators to avoid confusion by using another common abbreviation, TCE, shared with trichloroethane (methyl chloroform), trichloroethanol, and other compounds.

To give perspective to the evaluation of the health hazards from exposure to TCI, Chapter 3 provides basic background information of the physical and chemical properties of TCI; its production, sources and emissions; atmospheric transport, transformation and fate; ambient air, water, and soil concentrations; analytical methods for detecting and quantifying; and ecological effects. The large quantities of TCI continually emitted into the atmosphere have resulted in a mixing rate in the troposphere of the northern hemisphere of 11 to 17 ppt, a residence time of 2 weeks, with extensive photooxidative decomposition into two principal products of health concern, phosgene and dichloroacetyl chloride. In water, TCI has also been found in significant concentration, up to 403 ppb in raw surface and subsurface water and up to 21 ppb in finishing water. The major route of removal of TCI from water is volatilization, and its estimated half-life in surface waters ranges from 3 hours for rapidly moving shallow streams to 10 days or longer for ponds and lakes. These common sources of exposure to TCI and their potential to adversely affect human health are also reviewed in Chapter 3.

TCI is readily absorbed into the body through the lungs by exposure to its vapors and via the gastrointestinal tract after oral ingestion of contaminated water and food. In the body, it is extensively metabolized, principally in the liver, and because of its high lipid solubility it has a long residence time in adipose tissue. The distribution, storage, and biotransformation of

TCI to epoxides and other reactive metabolites underlies its human toxicity, including teratogenic, mutagenic and carcinogenic potentials. The toxicity of TCI is also substantially enhanced by prior or concurrent exposure to certain clinical drugs, alcohol and other xenobiotics. These aspects of the disposition and fate of TCI in the human body, as well as the methods of determining and quantifying exposure intensity in humans, are discussed in Chapter 4.

TCI at high air concentrations (>1000 ppm for several minutes or longer) is a potent central nervous system depressant and anesthetic, a cardiac depressant with a propensity to induce cardiac arrhythmias, and an hepatic and renal toxin. At lower-level exposure (50 to 500 ppm for several minutes or longer), TCI exerts behavioral and psychological effects that affect manual and mental performance. The adverse effects of low-level, chronic exposure to TCI have not been as extensively studied in animals or man. Studies of these adverse health effects of TCI at different levels of exposure are reviewed and evaluated in Chapter 5. Synergistic and antagonistic interactions of TCI with other drugs and xenobiotics are also identified in this chapter.

Controversial and major issues of concern with TCI exposure are the human teratogenic and carcinogenic potentials of this chlorinated hydrocarbon solvent. TCI readily crosses the placental barrier. The evidence that TCI is teratogenic, especially at low levels, is weak and the studies are few at present; however, some of the available evidence is suggestive of a damaging effect on fetal development. The studies relating to teratogenicity, fetal toxicity and reproductive effects are reviewed and evaluated in Chapter 6.

The appendix presents in detail the evidence for the cancer causing activity of TCI (CAG Report, 1981[2]). Chapter 7 summarizes this evidence from the currently available mutagenicity studies, animal carcinogenicity studies, and epidemiological data. The controversy provoked by clouded interpretation of studies with technical grade TCI containing potentially mutagenic and carcinogenic epoxide compounds as chemical stabilizers is also presented in this chapter. The Carcinogen Assessment Group Report, which includes the Reproductive Effects Assessment Group Report on trichloroethylene, is presented in its entirety in the appendix and provides more detailed description and discussion of this information.

---

[2] The Carcinogen Assessment Group's, Carcinogen Assessment of Trichloroethylene, August 1981.

3. BACKGROUND INFORMATION

The objective of this chapter is to provide a comprehensive information base relevant to the effects on human health from all modes of exposure to trichloroethylene. Reviewed in this chapter are the chemical and physical properties of trichloroethylene (TCI), production and use, environmental fate and transport, environmental levels of exposure (air and water), quantitative methods of measurement, and the ecological effects of TCI.

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Trichloroethylene (TCI) is a colorless, volatile liquid that is miscible with a variety of solvents. Its evaporation rate is three times that of ethyl ether (Waters et al., 1977). Some of its important physical properties are shown in Table 3-1.

TABLE 3-1. PHYSICAL PROPERTIES OF TRICHLOROETHYLENE

Molecular weight	131.39
Boiling point	87°C
Vapor pressure	94 mm Hg @ 30°C
Vapor specific gravity	4.55 at boiling point (air = 1)
Solubility @ 20°C	0.107 gm percent

At standard temperature and pressure (25°C, 1 atmosphere), one part-per-million (ppm) of the vapor is equivalent to 5.38 mg/m^3.

Trichloroethylene is photochemically reactive and autooxidizes upon catalysis by free radicals (McNeill, 1979). Autooxidation is greatly accelerated by high temperatures and exposure to ultraviolet radiation (McNeill,1979). Some of its degradation products, e.g., hydrochloric acid (HCl), are corrosive to metals. These decomposition products, some of which are highly toxic, include dichloroacetyl chloride, phosgene, carbon monoxide, hexachlorobutene, and HCl (McNeill, 1979.)

Decomposition is catalyzed when TCI comes in contact with aluminum metal. The HCl produced reacts with aluminum to yield aluminum chloride ($AlCl_2$) which catalyzes formation of hexachlorobutene (McNeill, 1979). Sufficient quantities of aluminum have been reported to cause violent decomposition of TCI (Metz and Roedy, 1949). While TCI is nonflammable under ordinary conditions, mixtures of TCI and oxygen will ignite at temperatures above 25.5°C when the TCI concentration is between 10.3 and 64.5 percent (Jones and Scott, 1942).

To inhibit its decomposition, stabilizers are added to commercial formulations of TCI (McNeill, 1979). Commonly used stabilizers in vapor degreasing grades of TCI are neutral inhibitors and free radical scavengers (McNeill, 1979). Epoxides (including epichlorohydrin, a known carcinogen) are added to scavenge free HCl or $AlCl_3$. Antioxidants are usually added in quantities less than 1 percent by weight (Hardie, 1964). Fuller (1976) reported that inhibited grades of TCI are stable up to 130°C in the presence of air, moisture, light, and common construction metals. At higher temperatures, inhibitors are less effective and corrosion of metals can result. Stabilizers used in TCI formulations are shown in Table 3-2.

Trichloroethylene decomposes at temperatures up to 700°C. Substances found in the decomposition mixture include dichloroethylene, tetrachloroethylene, carbon tetrachloride, chloroform, and methyl chloride (Hardie, 1964). Formation of phosgene, a highly toxic gas, was observed when TCI came in contact with iron, copper, zinc, or aluminum over the temperature range 250°C to 600°C (Sjoberg, 1952). When TCI came in contact with zinc at 450°C, 69 mg phosgene per gram of TCI was produced.

TABLE 3-2. STABILIZERS IN TRICHLOROETHYLENE FORMULATIONS (Beckers, 1972; U.S. EPA, 1975; Hardie, cited in: Kirk Othmer, 1975; Schlossburg, 1980)

Amyl alcohol
Propanol
Diethylamine
Triethylamine
Dipropylamine
Diisopropylamine
Diethanolamine
Morpholine
N-Methylmorpholine
Aniline
Acetone
Ethyl acetate
Borate esters
Ethylene oxide
1,2 Propylene oxide
1,2 Epoxy butene
Cyclohexene oxide
Propylene oxide
Butadiene dioxide
Styrene oxide
Pentene oxide
2,3 Epoxy 1-propenol
3, Methoxy-1,2 epoxy propane
3, Ethoxy-1,2 epoxy
Sterarates
2-Methyl-1,2 epoxy propanol
Epoxy cyclopentanol
Epichlorohydrin
Tetrahydrofuran
Tetrahydropyran
1,4-Dioxane
Dioxalane
Trioxane
Alkoxyaldehyde hydrazones
Methylethyl ketone
Nitromethanes
Nitropropanes
Phenol
o-Cresol
Thymol
p-tert-Butylphenol
p-tert-Amylphenol
Isoeuganol
Pyrrole
n-Methyl pyrrole
n-Ethyl pyrrole
(2-pyrryl) Trimethylsilane
Glycidyl acetate
Isocyanates
Thiazoles

Trichloroethylene is not readily hydrolyzed by water (McNeill, 1979). In the presence of strong alkali (e.g., sodium hydroxide) TCI can decompose to dichloroacetylene, an explosive, flammable, and highly toxic compound (Humphrey and McClelland, 1944). Noweir et al. (1973) recommended that TCI be stored in cans or dark glass bottles to minimize decomposition.

Because of its lipophilic properties, TCI can be stored in the body's adipose tissue. The relationships between such storage and manifestation of its toxic properties is discussed in Chapter 4. The unsymmetrical nature of the molecule and the electron-withdrawing effect of the chlorine substituents (leading to destabilization of the charge at the double bond) have been proposed as factors reponsible for the supposed mutagenicity of TCI (Bonse and Henschler, 1976).

Measurements of the lipophilic potential of TCI have been made by a number of investigators (Waters et al., 1977; Mapleson, 1963; Powell, 1947; Sato and Nakajima, 1979). Mapleson (1963) reported an oil/water partition coefficient of 960:1 at 37°C. Waters et al. (1977) reported the oil/water coefficient as 900:1. Recently Sato and Nakajima (1979) reported an olive oil/water partition coefficient of 552:1 at 37°C and an olive oil/blood partition coefficient of 76:1. The relationship of TCI to other compounds with respect to partition coefficients is shown in Table 3-3 (Sato and Nakajima, 1979).

TABLE 3-3. PARTITION COEFFICIENTS OF TRICHLOROETHYLENE AND OTHER CHLORINATED HYDROCARBONS (Sato and Nakajima, 1979)

Solvent	Oil/water	Oil/blood	Log P (partition coefficient)
Dichloromethane	21	16	1.32
Chloroform	115	39	2.06
Carbon tetrachloride	1444	150	3.16
1,2-Dichloroethane (EDC)	40	23	1.60
Methyl chloroform	383	108	2.58
Tetrachloroethylene	4458	146	3.65
Trichloroethylene	552	76	2.74

3.2 ENVIRONMENTAL FATE AND TRANSPORT

3.2.1 Production

Trichloroethylene (TCI) has been declining in production in recent years owing to restrictions on emissions (Federal Register, 1977), substitution by other solvents, and other factors. However, according to statistics published by the U.S. International Trade Commission, this overall decline from early 1970 production has greatly fluctuated from year to year. Table 3-4 shows that no trend has clearly been established for the production and consumption of TCI, taking into account erratic fluctuations in imports and exports.

TABLE 3-4. TCI PRODUCTION AND CONSUMPTION (Thousand Metric Tons)

Year	Production	Exports	Imports	Consumption[b]
1974	176	19.6	.6	157
1975	133	15.4	3.8	121
1976	143	16.1	7.0	134
1977	135	21.8	8.7	122
1978	136	15.8	9.4	130
1979	145	26.6	4.3	123
1980[a]	130			

[a] Estimated from preliminary USITC data
[b] Consumption = Production + Imports - Exports
Source: USITC 1975, 1976, 1977, 1978, 1979, 1980, SRI 1980

Recent estimates of world production of TCI were not available. Such figures for 1973 indicated 700,000 metric tons were produced (McConnell et al., 1975). About two-thirds was produced outside the United States.

In 1977, TCI was produced by five American manufacturers (SRI, 1978). The producers and production capacities are shown in Table 3-5.

TABLE 3-5. U.S. PRODUCTION CAPACITY FOR TRICHLOROETHYLENE (SRI, 1978)

Producer	Site	Annual capacity (metric tons)
PPG Industries	Lake Charles, LA	127,000
Dow Chemical	Freeport, TX	68,040
Diamond Shamrock	Deer Park, TX	24,948
Occidental Petroleum	Taft, LA	22,680
Ethyl Corp.	Baton Rouge, LA	18,144

Most of the TCI produced commercially is derived from ethylene or dichloroethane (ECD) (McNeill, 1979). Dichloroethane, produced by chlorination of ethylene, can be further chlorinated to TCI and tetrachloroethylene at 280° to 450°C. Catalysts include potassium chloride, aluminum chloride, fuller's earth, graphite, activated carbon, and activated charcoal (McNeill, 1979). Maximum conversion to TCI (75% of EDC feed) is achieved at a chlorine to EDC ratio of 1.7:1 (McNeill, 1979).

Oxychlorination of ethylene or EDC to TCI is achieved at 425°C and at 20 to 30 psi (McNeill, 1979). Common catalysts are mixtures of potassium and cupric chlorides. Conversion efficiency is between 85 and 90 percent.

3.2.2 Use

It has been estimated that from 80 to 95 percent of the TCI produced in the United States is used in the degreasing of fabricated metal parts (SRI, 1978; McNeill, 1979; Chemical and Engineering News, 1975). The remaining 5 to 20 percent is divided equally between exports and miscellaneous applications. Use in vapor degreasing is estimated to account for 87 percent of production according to industry trade statistics (Chemical Marketing Reporter, 1978).

SRI International (1978) forcasted a decline in consumption from the 1977 level at a rate of 5.5 percent compounded annually through 1982. They predicted a leveling in consumption to 90,000 metric tons by 1988. A similar decline, followed by a leveling in the market by 1983, was projected by others (Chemical Marketing Reporter, 1978). However, actual data from the USITC have not supported these forecasts. Consumption of TCI increased in 1978 and then declined in 1979 (Table 3-4). The data suggest that no clear trend has yet been established for either production or consumption of TCI.

In addition to its use as a vapor degreasing agent, TCI has a wide variety of other uses (Table 3-6). About 4,500 to 6,800 metric tons are consumed annually in the production of polyvinyl chloride in which TCI is used as a chain-transfer agent (McNeill, 1979).

TABLE 3-6. MISCELLANEOUS USES OF TRICHLOROETHYLENE
(McNeill, 1979; Waters et al., 1977)

Adhesive formulations
Paint-stripping formulations
Low-temperature heat-transfer medium
Carrier solvent in industrial paint systems
Solvent in textile dyeing and finishing

Uses of TCI that have been discontinued in the United States because of environmental and health restrictions include use as an inhalation anaesthetic (Page and Arthur, 1978; Waters et al., 1977), in fumigant mixtures (Farm Chemicals Handbook, 1976), and as an extractant in the decaffeination of coffee. Use in anaesthetic procedures appears to be continuing to some extent in other countries (Cundy, 1976).

Declining consumption of TCI from the early 1970's has been offset by the increased use of substitute solvent, e.g., methyl chloroform and methylene chloride (EPA, 1979).

3.2.3 Emissions

Because of its dipersive use pattern in degreasing operations, most of the TCI used is expected to be emitted to the atmosphere. There are no known natural sources of TCI. Graedel and Allara (1976) concluded that atmospheric chemical production of TCI was negligible. Recent emissions estimates (EPA, 1979) for 1976, pertaining to vapor degreasing, cold cleaning, and miscellaneous use categories suggest that 130,000 metric tons were discharged to the atmosphere.

Solvent emissions from vapor degreasing occur primarily during unloading and loading of the degreaser (Bucon et al., 1978). Daily emissions of a single spray degreasing booth may vary from a few pounds to 1,300 pounds (Bucon et al., 1978). In uncontrolled degreasing operations, as much as 2.8 kg/hour can be emitted (based on 200,000 pounds of metal cleaned per day) (Bucon et al., 1978).

McConnell et al. (1975) estimated that, for 1973, global emissions of TCI amounted to about 1 million metric tons.

Singh et al. (1979) estimated global emissions of TCI for 1977 at 435,000 metric tons ± 50 percent. Estimates were derived from production figures and usage patterns.

3.2.4 Persistence

Many processes that occur in the troposphere influence the potential for trichloroethylene (TCI) in ambient air to pose a hazard to human health. Once emitted into the troposphere, vertical and horizontal mixing occurs. Transport is highly dependent upon the length of time TCI remains in the troposphere, which is determined largely by the extent to which TCI reacts with hydroxyl free radicals (OH), the principal scavenging mechanism for TCI and many other halogenated compounds in the atmosphere. Recent estimates (Singh, 1977; Crutzen and Fishman, 1977) of the average OH concentration, supported by the results of field measurements (Campbell et al., 1979), suggest that they are lower than previously believed ($<1 \times 10^6$ molecules cm^{-3}). If true, survival of most halogenated compounds would be correspondingly longer. The tropospheric lifetime is related to the OH concentration by the following expression (where k is the rate constant of reaction):

$$t_{lifetime} \propto \frac{1}{k[g \cdot OH]}$$

Irrespective of the tropospheric lifetime, the nature of the transformation products resulting from the photochemical decay of TCI is of additional concern, particularly if these products pose hazards to human health.

The average northern hemisphere background mixing ratio is between 11 and 17 ppt (Singh et al., 1979b), which suggests that TCI is short-lived in the troposphere. Singh et al. (1979a) estimated a residence time of about 2 weeks, an estimate based upon a seasonally-averaged OH concentration of 4×10^5 molecules cm^{-3} and a rate constant (National Bureau of Standards, 1978), at $265^{o}K$, of 2.30×10^{-12}. Singh and coworkers (1979b) estimated that, given an ·OH concentration of 1×10^6 molecules cm^{-3}, 20 percent of the TCI in ambient air can be destroyed each day. Crutzen et al. (1978) estimated a residence time of 11 days for TCI. Derwent and Eggleton (1978) estimated the lifetime at 0.04 years (~ 15 days). The percentage of the ground level injection of TCI that was estimated to survive ·OH attack was 0.4 percent. The estimated percentage decrease in stratospheric ozone due to global TCI emissions at current rates was 0.04 (British Department of the Environment, 1979).

Seasonal variations in ·OH concentrations, important for longer-lived species, are not expected to play a significant role in the tropospheric survival of TCI. Altshuller (1980) calculated that in January (when ·OH levels and solar flux are low) 0.6 days would be required for the photochemical decomposition of 1 percent of the ambient TCI. In July (when ·OH levels and solar flux are high), only 0.07 days is required.

Trichloroethylene was observed to have a slow decomposition rate in dilute aqueous solution (Dilling et al., 1975). Its half-life (in the dark) was 10.7 months at ambient temperatures. When the solution was exposed to sunlight, 75 percent of the TCI decomposed in 12 months compared with 54 percent for the reaction in the dark. A correction for the amount of TCI that volatilized into the air space above the solution was not employed. On the other hand, Pearson and McConnell (1975) in their determinations of the hydrolytic decomposition extrapolated to zero volatilization. Their estimate was a half-life of 30 months.

The major route of removal of TCI from water is volatilization. Volatilization of TCI from water has been investigated by several authors. Dilling et al. (1975) has shown that the loss of TCI from an agitated dilute aqueous solution occurs exponentially with an evaporative half-life of 21 ± 3 minutes. Scherb (1978) measured the volatilization of TCI from an aeration channel in a wastewater treatment plant and found the half-life to be 3 hours. The rate of volatilization of TCI from surface waters in the environment has been reported by Zoeteman et al. (1980). TCI was found to have a half-life of 1-4 days in the Rhine River and 30-40 days in a tidal estuary. Smith et al. (1980) has shown that the rate of volatilization of TCI and other low molecular weight compounds from various bodies of waters is a function of reaeration rates. From the work of Smith et al., it is estimated that the half-life for TCI in surface waters ranges from 3 hours for rapidly moving shallow streams to 10 days or longer for ponds and lakes, Table 3-7.

TABLE 3-7. ESTIMATED HALF-LIFE OF TCI IN SURFACE WATERS

	T 1/2 (days)
Pond	11
Lake	4-12
River	1-12

Source: Calculated from information in Smith et al. (1980).

3.2.5 Degradation

The transformation products of the decay of TCI have been investigated in chamber studies simulating atmospheric conditions. Two principal transformation products have been observed: phosgene and dichloroacetyl chloride. Dahlberg (1969) observed that both were produced when TCI was photooxidized in air. Gay et al. (1976) also found formyl chloride in addition to phosgene and dichloroacetyl chloride when TCI was photooxidized in the presence of nitrogen dioxide (NO_2). In this system, dichloroacetyl chloride, identified by long-path infrared spectroscopy, was the principal product.

Lillian et al. (1976), finding phosgene formation from the photooxidation of the TCI congener, tetrachloroethylene, considered that phosgene formation also was likely from other chloroethylenes. Singh (1978) reported that ·OH-catalyzed photodecomposition of TCI, such as occurs in the troposphere, can result in phosgene as the principal transformation product.

Under simulated atmospheric conditions in the presence of nitric oxide (NO) and nitrogen dioxide (NO_2), TCI (10 ppm) was very short-lived (less than 3.5 hours) (Dilling et al., 1976). The high reactivity of TCI and its catalytic role in the formation of photochemical smog have been recently discussed (EPA, 1978a; National Academy of Sciences, 1977). Free radicals (e.g., $\cdot RO_2$), are formed when TCI is photodecomposed and these, in turn, react with NO by oxidizing it to NO_2. According to the following reaction sequence, NO_2 results in the formation of ozone (O_3):

$$(1)\quad NO_2 \xrightarrow{h\nu} NO + O$$

$$(2)\quad O + O_2 + M \text{ (third body)} \longrightarrow O_3 + M$$

Inasmuch as chamber studies simulate some atmospheric reactions believed to occur in the environment, further studies of greater complexity would be needed to determine the extent and pathways by which TCI is photodecomposed. Phosgene and dichloroacetyl chloride, while likely to be formed in the troposphere, also are subject to transformation and scavenging processes.

While phsogene is a highly toxic gas, little is known of its effects upon long-term exposure at ambient levels (NIOSH, 1976). Singh (1976), based on the studies of Gay et al. (1976), estimated that emissions of tetrachloroethylene and TCI, in 1976, could result in the formation of about 0.3 x 10^6 metric tons of phosgene annually. Under adverse meteorological conditions, higher concentrations of phosgene could occur in and around urban centers, since it is only slowly removed from the atmosphere. Similarly, the results obtained by Gay et al. (1976) with regard to dichloroacetyl chloride, another highly toxic gas, suggested to Singh (1976) that levels comparable to or greater than phosgene would also be present.

3.3 LEVELS OF EXPOSURE

3.3.1 Analytical Methodology

Of the analytical methods and procedures available for detecting and quantitating halogenated hydrocarbons present in ambient air, water, soil, foods, etc., two systems are commonly used: gas chromatography/mass spectrometry (GC-MS) and gas chromatography with electron capture detection (GC-EC). Each system has the capability of detecting TCI concentrations as low as a few parts-per-trillion by volume (ppt). The usefulness of GC-EC is that the apparatus can be used in the field to provide quasi-continuous measurements by intermittent sampling (every 15 to 20 minutes). Such use can circumvent decomposition that results when samples of TCI are stored for long periods prior to assay (Pellizzari, 1974; Lovelock, 1974b).

3.3.1.1 Gas Chromatography with Electron Capture Detection--The GC-EC methodology has been reviewed by Pellizzari (1974) and by Lovelock (1974b). The EC detector is specific in that halogenated compounds are quantified while non-halogenated hydrocarbons are not detected. Thus, high background levels of non-halogenated hydrocarbons in ambient air or water samples do not interfere with measurements of halogenated compounds. In a complex mixture in which several compounds may have similar retention times, alteration of the operating parameters of the GC-EC system will usually provide separation of the components.

In the method developed by Rasmussen et al. (1977), the detection limit for TCI was 0.3 ppt. With four samples of ambient air, the percent standard deviation was 23.0 by electronic area integration (+5 percent by peak height measurement). The system, which employs a freezeout concentration loop, has the capability of measuring various halocarbons in 500 ml aliquots of air. Total analysis time is 40 minutes. The GC column consisted of a 6 mm x 3 m stainless steel column packed with 10 percent SF-96 on 100/120 mesh-Chromosorb W.

A similar analytical procedure was employed by Harsch and Cronn (1978) to measure levels of halocarbons in 30 ml samples of low-pressure stratospheric air. For TCI the detection limit was 1 ppt and the precision (percent standard deviation) for 10 samples with a mixing ratio of 22 ppt was 10 percent.

Gas chromatography with electron capture detection also has been the method of choice by Singh and coworkers for measuring the ambient air levels of a wide variety of halocarbons. Singh et al. (1979a) maintained the EC detector at 325°C. The GC column used was 6 ft x 1/8 inch stainless steel containing 20 percent SP2100, 0.1 percent Carbowax 1500 on 100/120 mesh Supelcoport. Singh et al. (1979b) recommended two columns for separation of TCI: (a) silicone oil 10 percent DC 200 on 30/60 mesh Chromosorb W in a 6 ft x 1/4 inch stainless steel column or (b) 20 percent SP-2100 on 80/100 mesh Supelcoport in a 15 foot x 1/8 inch column.

The applicability of GC-EC to analysis of TCI and other halohydrocarbons purged from water samples has been discussed by Bellar et al. (1979). Preconcentration on a Tenax® and silica gel trap was recommended.

Singh et al. (1979a) avoided the use of Tenax® traps for preconcentrating organics in air samples because of artifacts. It was thought that oxygen or ozone was oxidizing the Tenax® monomer and producing electron absorbing oxygenated species, thus interfering with EC detection of the organics of interest.

Makide et al. (1980) used GC, coupled with constant current ECD, to measure TCI in ambient air at sites in Japan. The reported detention limit was < 0.2 ppt ($< 1.36 \times 10^{-6}$ mg/m^3). Sampling canisters were made of stainless steel and were polished electrochemically. Preconcentration was carried out on a silicone OV-101 column at - 40°C. Column temperature was raised at a rate of 5°C per minute up to 70°C for separation of components.

A direct head-space technique for use with EC detection has been reported by Piet and coworkers (1978). This technique avoids the need for preconcentration traps when water samples are purged. An overall detection limit below 1 μg/l was reported. With isothermal operation of the GC, a detection limit for TCI of 0.05 μg/l was reported. An OV225 capillary column was used.

Dietz and Singley (1979) recently described a headspace method applicable to EC detection of volatile organics from water samples. A standard deviation of 5 percent was reported for routine analyses of drinking, natural, and industrial waters. Concentrations ranging from 0.1 ppb to several ppm of TCI were detected. In this method, the water sample is sealed in a vial until organics equilibrate between the water and vial headspace. A major advantage is that only relatively volatile, water-insoluble compounds tend to partition in the headspace. In a comparison of this method with the purge and trap technique, Dietz and Singley (1979) obtained comparable results. However, the chromatograms from the purge-trap method were of poorer quality. The headspace method was recommended for routine sample analyses of volatile halohydrocarbons.

3.3.1.2 Gas Chromatography-Mass Spectrometry--While GC-EC relies on retention time and ionization efficiency for compound identification, the equally sensitive GC-MS system identifies compounds by their characteristic mass spectra. However, the expense, bulk, and demands upon the operator preclude routine use of GC-MS in field measurements. Often, GC-MS is used to verify results obtained by GC-EC.

Comparisons between the precision afforded by GC-MS versus GC-EC have been made by Cronn and co-workers (1976). In these comparisons, the detection limit was defined based on a peak height three times the noise level with a 75-ml sample loop. With TCI at an average mixing ratio of 128 ppt, the detection limit (GC-MS) was 23 ppt (with 3 doped samples of zero air). The percent standard deviation was 10. More recent data from Cronn and Harsch (1979) show a slightly lower detection limit (16 ppt) for a 100 ml freezeout sample. The sensitivity of the GC-MS technique was achieved using direct coupling of the gas chromatograph and mass spectrometer and selective ion monitoring (at mass 95 for TCI).

Pellizzari and Bunch used Tenax® GC to absorb TCI and other halogenated hydrocarbons from ambient air. Recovery was made by thermal desorption and helium purging into a freezeout trap. The estimated detection limits when high resolution GC-MS is used, is 1.92 ppt (0.01×10^{-3} mg/m^3). Accuracy was reported at ± 30 percent. Included among the inherent analytical errors were (1) the ability to accurately determine the breakthrough volume, (2) the percent recovery from the sampling cartridge after a period of storage, and (3) the reproducibility of thermal desorption from the cartridge and its introdution into the analytical system. To minimize loss of sample, cartridge samplers should be enclosed in cartridge holders and placed in a second container that can be sealed, protected from light, and stored at 0°C. The advantages reported for Tenax® include (1) a low water retention, (2) a high thermal stability and (3) low background levels (Pellizzari, 1974; Pellizzari, 1975; Pellizzari, 1977; Pellizzari et al., 1976).

Resolution of TCI from other hydrocarbons was reported to be effective when Carbopak C-HT, a graphitized thermal carbon black treated with hydrogen at 1,000 °C was used as the adsorbent in a GC column (Knoll et al., 1979). Flame ionization was used in detection. Interferences between TCI and ethylene dichloride was reported with Porapak T.

Gas chromatography-mass spectrometry has been more widely used in measurements of volatile organic compounds in aqueous samples. In this regard, gas purging followed by trapping on an absorbent has been extensively used in combination with GC-MS detection. A recent review of this system, as applied to TCI and other volatile organics, has been made by Bellar et al. (1979). A combination of Tenax® GC(60/80 mesh) and silica gel was recommended. Purging was accomplished at a water temperature of about 22°C. In general, the method is applicable to compounds that have a low solubility in water and a vapor pressure greater than water at ambient temperatures. For well-defined samples when the probability of unexpected compounds is low, the EC detector could be used. The detection limit of this system was reported to be in the 0.1 to 1 µg/l range. Desorption onto the head of the column can be accomplished in one step by heating.

3.3.2 Calibration

Both static and dynamic procedures have been used to calibrate the instrumentation used for measurement of TCI. Due to the very low levels of TCI found or expected in ambient air, the generation of known low-level concentrations of TCI is critical in instrument calibration.

Singh and coworkers (1979a, 1977) employed permeation tubes to calibrate their GC-EC instrumentation. A tube constructed of FEP Teflon® (wall thickness of 0.03 inches) was found to be satisfactory for TCI. Errors in the permeation rate were less than 5 percent (Singh et al., 1979a). It was considered the best, most accurate means of generating primary standards. Since primary standards could not be used in field operations, secondary standards were generated and compared against the primary standards. The permeation rate at the 95 percent confidence level was 246.0 ± 20.1 mg/min (Singh et al., 1977).

Primary and secondary standards prepared by multiple dilutions were used by Cronn and coworkers (1976, 1977,a & b).

3.3.3 Sampling of Ambient Air and Water

Common approaches used to sample ambient air for trace gas analysis are presented below (National Academy of Science, 1978).

(1) Pump-pressure samples: A mechanical pump is used to fill a stainless steel or glass container to a positive pressure relative to the surrounding atmosphere.

(2) Ambient pressure samples: An evacuated chamber is opened and allowed to fill until it has reached ambient pressure at the sampling location. If filling is conducted at high altitude, the container may easily become contaminated when returned to ground level.

(3) Adsorption on molecular sieves, activated charcoal, or other adsorbents.

(4) Cryogenic samples: Air is pumped into a container, liquified and a partial vacuum is created which allows more air to enter. This method allows for the collection of several thousand liters of air.

Singh and coworkers (1979b) employed electropolished stainless steel vessels in their sampling of tropospheric air. Vessels were checked for background contamination until it was reduced to less than 2 to 3 percent of the expected background concentration of a given trace substance. Typically, vessels that maintained positive pressure were not affected by room contamination.

Sampling and storage of ambient air collected at stratospheric altitudes presents more of a problem. Due to the low pressures in the sampling vessels, contamination may easily result when vessels are returned to ground level. Cronn and Robinson (1978) and Harsch and Cronn (1978) reported that contamination of low-pressure stainless steel vessels precluded use of data for TCI. Whole-air samples were collected at stratospheric altitudes. Contamination occurred during the sample-transfer procedure due to high mixing ratios of halocarbons in laboratory air.

In order to overcome contamination of low-pressure samples, Harsch and Cronn (1978) developed a low-pressure sample-transfer technique. A vacuum transfer procedure employing a freeze-out loop (Rasmussen et al., 1977) was found to yield results with sufficient sensitivity and with minimal contamination problems. A leak-tight system was considered essential. System components were silver soldered.

Sample collection, handling, and preservation of water samples have been discussed by Kopfler et al. (1976) and by Bellar et al. (1974). Narrow-mouth vials with a total volume in excess of 50 ml were considered acceptable (Bellar et al., 1974). It was recommended that the vials be filled to overflowing and each covered with a Teflon®-faced silicone rubber septum. Due to the presence of chlorine in some drinking water supplies and the chlorination of organic materials, an increase in the concentration of some halohydrocarbons may occur also during storage (Kopfler et al., 1976).

Bellar et al. (1974) reported that free chlorine can result in the formation of TCI and other halohydrocarbons. In measurements of TCI in water from a sewage-treatment plant, they found an increase of 1.2 μg TCI/l in the water after chlorination. Dietz and Singley (1979) collected water samples in 125 ml serum vials that had been cleaned as follows: detergent wash, distilled water rinse, dichromic acid wash, distilled water rinse, acetone rinse, hexane rinse, acetone rinse, distilled water rinse, and oven-drying at 150°C. Vials were completely filled and then sealed with Teflon®-faced septa and aluminum crimp seals. Samples were stored at 4°C. Minimum loss occurred during storage at 4°C up to 28 days.

The stability of stored samples of TCI present in ambient air or breath samples from exposed individuals has been investigated by Pasquini (1978). The mean percent loss of TCI in alveolar breath samples collected in glass tubes at room temperature was 59.3 ± 15.3 percent over 169 hours. The mean decay rate of TCI in dilution air samples was 4.7 ± 3.2 percent over the same time interval. Moisture, temperature, and tube surface and condition can greatly alter vapor retention. For TCI, tubes stored at 37°C had high vapor retention rates. When breath was exhaled into the tubes at 37°C, water vapor was not condensed from the sample. Additional experiments with TCI indicated that siliconized tubes showed a lower decay rate than non-siliconized tubes.

Renberg (1978) judged XAD-4 as the most suitable of several possible adsorbents for volatile halogenated hydrocarbons in water, including TCI. This method results in an extract concentrated enough for both chemical determination and small scale biological tests.

Kummert et al. (1978) have suggested a direct aqueous injection high-pressure liquid chromatography technique for analysis of TCI in natural waters.

The technique has been successfully applied to an actual water pollution case for tetrachloroethylene. The detection limit for TCI was reported as 1 μmol l^{-1} for a 10 ml sample.

3.3.3.1 Air-Mixing Ratios--Measurements of ambient air mixing ratios of TCI have been made at a variety of sites in the United States and abroad. As shown in Table 3-8, urban areas have mixing ratios in the low ppb range.

Singh et al. (1979) reported an average mixing ratio in the northern hemisphere of 11 to 17 ppt with the average in the southern hemisphere of less than 3 ppt. Over the Pacific Northwest, an average tropospheric mixing ratio of 20 ± 3.9 ppt was found (Cronn et al., 1977a). Harsch (1977) reported levels between 22 ppb and 8.7 ppb in more than 20 different indoor environments.

3.3.3.2 Water--Trichloroethylene has been detected in a number of raw water sources, finished drinking waters, and subsurface waters in the United States. The occurrence of TCI in waters of the United States has been recently reviewed by the U.S. Environmental Protection Agency (1979).

(1) Drinking water

Dowty et al. (1975) detected TCI in finished drinking water in the New Orleans area. It was reported to pass through the water treatment plant without alteration. It was also reported to be incompletely removed after passage of water through a charcoal filtering unit. Detection was made by gas chromatography-mass spectrometry (GC-MS).

The most comprehensive surface water monitoring effort for TCI to date (Ewing et al., 1977) has shown TCI to be a common low-level (ppb) contaminant of surface waters in the vicinity of urban/industrial areas. TCI was reported at concentrations of 1 ug/l (ppb) or greater in 72 of 179 surface water samples from U.S. urban/industrial areas from 23 states (Table 3-9). Ewing also reported samples of drinking water which were not included in this total.

TABLE 3-8. AMBIENT AIR MIXING RATIOS OF TRICHLOROETHYLENE

Location	Type of site	Date of measurement	Mixing ratio ppb maximum	minimum	average	Reference
ARIZONA						
Phoenix	Urban	4/23-5/6 1979	3.0696	0.0117	0.4835 ± .5869	Singh et al., 1981
ARKANSAS						
Helena	Remote	11/30/76	≤ 0.03	≤ 0.03	≤ 0.03	Battelle, 1977
CALIFORNIA						
Stanford Hills	Background subject to urban transport	11/23-11/30 1975	3.09	0.006	0.093 ± 0.32	Singh et al., 1979a
Los Angeles	Urban	4/28-5/4 1976	1.77	0.026	0.315 ± .310	*Ibid*
Los Angeles	Urban	4/9-4/21 1979	1.702	0.363	0.399 ± .302	Singh et al., 1981
Point Reyes	Marine	12/1-12/12 1975	0.064	0	0.024 ± 0.014	Singh et al., 1979
Palm Springs	Urban	5/5-5/11 1976	0.446	0.006	0.035 ± 0.050	*Ibid*
Yosemite	High altitude	5/12-5/18	0.022	0.008	0.015 ± 0.003	*Ibid*
Mill Valley	Background subject to urban transport	1/11-1/27 1977	0.090	0.009	0.026 ± 0.019	*Ibid*
Riverside	Urban	4/25-5/4 1977	0.476	0.057	0.266 ± 0.094	*Ibid*

TABLE 3-8. (Continued)

Location	Type of site	Date of measurement	Mixing ratio ppb			Reference
			maximum	minimum	average	
Badger Pass	High altitude	5/5-5/13 1977	0.016	0.001	0.012 ± 0.002	Ibid
Point Arena	Marine	5/23-5/30 1977	0.012	0.010	0.011 ± 0.001	Ibid
Point Arena	Marine	8/30-9/5 1978	0.034	0.008	0.017 ± 0.007	Ibid
San Jose	Urban	8/21-8/27 1978	2.192	0.048	0.417 ± 0.425	Ibid
Oakland	Urban	6/28-7/10 1979	1.5582	0.0141	0.1876 ± 0.2697	Singh et al., 1981
DELAWARE						
Delaware City	Urban	7/8-7/10 1974	0.56	< 0.05	0.35	Lillian et al., 1975
LOUISIANA						
Baton Rouge/Geismar/ Plaquemine	Urban	fall, 1978	-	trace	0.026	Pellizzari et al., 1979
MARYLAND						
Baltimore	Urban	7/11-7/12 1974	< 0.05	< 0.05	-	Ibid
MICHIGAN	Urban	3/6/75	0.09	0.03	0.05	Russell and Shadoff, 1977
NEW JERSEY						
Seagirt	Ocean shore	6/18-6/19 1974	2.8	< 0.05	0.26	Lillian et al., 1975
Sandy Hook	Background subject to urban transport	7/2-7/5 1974	0.8	< 0.05	0.34	Ibid

TABLE 3-8. (Continued)

Location	Type of site	Date of measurement	Mixing ratio ppb			Reference
			maximum	minimum	average	
NEW JERSEY (continued)						
Bayonne	Urban	March-Dec. 1973	8.8	< 0.05	0.92	Ibid
Boundbrook/ Rahway/Passaic	Urban	Sept. 1978	32.3	trace	23	Pellizzari et al., 1979
NEW YORK						
NYC	Urban	6/27-6/28 1974	1.1	0.11	0.71	Lillian et al., 1975
Whiteface Mtns.	High altitude	9/16-9/19 1974	0.35	< 0.05	0.10	Ibid
Niagara Falls/Buffalo	urban	fall, 1978	0.11	0.05	0.10	Pellizzari et al., 1979
OHIO						
Wilmington	Urban	7/16-7/26 1974	0.63	< 0.05	0.19	Ibid
NEVADA						
Reese River	High altitude	5/14-5/20 1977	0.016	0.001	0.012 ± 0.002	Singh et al., 1979
KANSAS						
Jetmar	Remote	6/1-6/7 1978	0.021	0.007	0.013 ± 0.004	Ibid
TEXAS						
Houston/Deer Park/ Pasadena	urban	fall, 1978	0.9	trace	0.06	Pellizzari et al., 1979
WASHINGTON						
Pullman	Rural	Dec. 1974- Feb. 1975	-	-	< 0.005	Grimsrud and Rasmussen, 1975

TABLE 3-8. (Continued)

Location	Type of site	Date of measurement	Mixing ratio ppb maximum	minimum	average	Reference
WASHINGTON (continued)						
Pacific Northwest	Background high altitude (4.6 → 14.6 km)	March 1976	-	-	0.020 ± 0.0039	Cronn et al., 1977a
PACIFIC OCEAN						
(lat 37°N)	Marine	April 1977	-	-	0.0124	Cronn et al., 1977b
PANAMA CANAL ZONE						
(lat 9°N)	Background	July 1977	-	-	0.015	Cronn and Robinson, 1978
NORTH ATLANTIC OCEAN	Marine	Oct. 1973	-	-	< 0.005 ± 0.0657	Lovelock, 1974a
WESTERN IRELAND	Background	June-July 1974	-	-	0.015 ± 0.0121	Ibid
ENGLAND	Rural	March 1972	.005	4×10^{-4}	.002	Murray and Riley, 1973a
JAPAN						
Tokyo	Urban	9/10-10/27 1975	47.1	0.8	3.5 ± 3.7	Tada et al., 1976
Various sites	remote	Summer, 1979	0.038	0.005	0.014 ± 0.010	Makide, et al., 1980
WESTERN EUROPE	Mixed	1976	8.5	< 0.02		Correia et al., 1977

TABLE 3-9. MEAN LEVELS OF TCI IN SURFACE WATERS[a]

	Surface water samples			Mean levels (ug/1)	
State	Positive for TCI	Total	Percent positive for TCI	All samples[b,c]	Positive samples only
Alabama	1	7	14	0.5	1.0
Delaware	4	10	40	1.2	2.3
Illinois	17	25	68	0.6	3.2
Indiana	1	4	25		1.0
Kentucky	1	3	33	0.7	1.0
Louisiana	1	7	14	0.6	1.0
Michigan	1	8	13	23.9	188
Minnesota	2	9	22	1.9	7
New Jersey	14	15	93	4.0	4.3
New York	10	20	50	1.6	2.6
Pennsylvania	14	21	67	2.3	3.2
Tennessee	1	6	17	0.9	3.0
Texas	4	8	50	1.2	1.8
West Virginia	1	3	3	0.7	1.0
All Sites	72	179	40	2.7	5.9

[a] Source: Ewing et al. (1977); drinking water samples not reported in these data.

[b] Mean levels were calculated by substituting a value of one-half the reporting limit of 1 ppb (1 ug/1) for samples reported as having non-detectable (i.e., less than 1 ug/1).

[c] Levels of TCI in sample from the following states (number of sites per state in parentheses) were reported as not detectable (i.e., less than 1 ug/1):

California (9)
Iowa (2)
Mississippi (1)
Missouri (3)
North Carolina (1)
Oregon (3)
Washington (2)
Wisconsin (6)

A concentration of 8.5 ppb was found in the drinking water of the town of Grand Island, New York (Coleman et al., 1976) using headspace gas chromatography coupled with electron capture detection (GC-EC). In 10 lake water samples from this area, an average concentration of 11.9 ppb ± 2.5 percent was found.

Coleman et al. (1976) detected TCI in the drinking water of four cities in 1975. The concentrations were: 0.1 ppb (Cincinnati); 0.3 ppb (Miami); <0.1 ppb (Ottumwa, Iowa); and 0.5 ppb (Philadelphia). No TCI was detected in drinking water samples for Seattle. Identification and quantification were made by GC-MS using the purge/trap technique.

As evidenced by the differences reported in the individual studies above, the frequency of contamination and the amount of TCI found in potable waters depends in part upon the source of water being monitored.

A total of 133 cities using surface water supplies have been sampled during federal surveys. Thirty-two percent of the finished waters serving these cities were found to contain TCI. The concentration of TCI in these water supplies ranged from 0.06 to 3.2 ug/l. The average concentration in positive water supplies was 0.47 ug/l. Thirty percent of all supplies contained a level of TCI which was below 1 ug/l. The remaining two percent of the supplies contained a concentration between 1 to 4 ug/l.

A total of 25 cities that draw their water from underground sources were sampled for TCI during the NOMS, NORS, SRI and EPA Region V surveys. Thirty-six percent of these finished waters were found to contain detectable concentrations of this chemical. The median level detected was 0.31 ug/l with a range from 0.11 to 53.0 ug/l.

The Community Water Supply Survey examined an additional 330 ground water systems selected randomly from across the U.S. Four percent of these systems were found to contain a detectable level of trichloroethylene. Seventy percent of these detectable levels of TCI were between 0.5 to 5 ug/l. TCI was found in finished water from small and large systems alike. For further information on levels of TCI in drinking water and exposure of the U.S. populace to these levels, the reader is advised to consult the U.S. EPA exposure assessment report on volatile organics in drinking water (1981a).

In analyses of drinking waters from sites near producer and user facilities and from a background site, Battelle found the following concentrations in 1977: 19 ppb (Freeport, Texas); 0.4 ppb (Baton Rouge, Louisiana); 0.1 ppb (Lake Charles, Louisiana); 32 ppb (Des Moines, Iowa); and 22 ppb (Helena, Arkansas).

(2) Rivers, treatment plant effluents

Up to 403 ppb TCI was detected by Battelle in surface waters near specific manufacturing facilities (1977). Trichloroethylene was detected in both industrial wastewater and river water receiving the waste (Jungclaus et al., 1978).

Murray and Riley (1973a) detected TCI in surface sea water in the eastern Atlantic ocean.

Bellar et al. (1974) found an increased concentration of TCI after chlorination treatment of the effluent from a sewage-treatment facility. Before chlorination, the concentration detected by GC-MS was 8.6 ppb; after chlorination, the concentration increased to 9.6 ppb.

Case reports describing TCI contamination of well waters and water supplies have been reviewed elsewhere (EPA, 1979). Concentrations as high as 330 ppb have been reported.

Correia et al. (1977) measured TCI in river, canal, and sea waters that receive effluent from production and user sites. Values ranged from 0.02 to

74 μg/l (ppb w/w), showing the effect of these additions on background levels. Dilution effects and losses to the surrounding air were noted.

3.3.3.3 Soil and Sediment--Battelle (1977) reported the presence of TCI in soil samples in the vicinity of producers and users at levels up to 5.6 ppb. Trichloroethylene was detected in the soil in the Love Canal area in Niagara Falls, New York (EPA, 1979).

Pearson and McConnell (1975) found up to 9.9 ppb in sediment from Liverpool Bay, England. Murray and Riley (1973b) found TCI in river mud.

In freshwater sediment taken near the Hooker Chemical facility at Taft, Louisiana, up to 300 ppb TCI was detected (Battelle, 1977).

3.4 ECOLOGICAL EFFECTS

Trichloroethylene (TCI) has been tested for acute toxicity in a limited number of aquatic species. This section presents observed levels that were reported to result in adverse effects under laboratory conditions. Such parameters of toxicity are not easily extrapolated to environmental situations. Test populations themselves may not be representative of the entire species in which susceptibility of various lifestages to the test substance may vary considerably.

Guidelines for the utilization of these data in the development of criteria levels for TCI in water are discussed elsewhere (EPA, 1980).

The toxicity of TCI to fish and other aquatic organisms has been gauged principally by flow-through and static testing methods (EPA, 1980). The flow-through method exposes the organism(s) continuously to a constant concentration of TCI while oxygen is continuously replenished and waste products are removed. A static test, on the other hand, exposes the organism(s) to the added initial concentration only. Both types of tests are commonly used as initial indications of the potential of substances to cause adverse effects.

Alexander et al. (1978) used both flow-through and static methods to investigate the acute toxicity of several chlorinated solvents, including TCI, to adult fathead minnows (Pimephales promelas). Studies were conducted in accordance with test methods described by the U.S. Environmental Protection Agency (1975). The lethal concentration of each solvent tested to produce 50 percent mortality (LC50) was lower in the flow-through as opposed to the static-type experiment. The flow-through method was considered the optimal of the two for volatile solvents such as TCI. The 96-hour LC50 concentration for TCI was 40.7 mg/l in the flow-through test. The 95 percent confidence band was between 31.4 and 71.8 mg/l. Of four solvents tested (perchloroethylene, methylene chloride, methylchloroform, and TCI), TCI had the second highest toxicity (after perchloroethylene).

In acute static experiments with bluegills, TCI was reported to have a 96-hour LC50 value of 44.7 mg/l (EPA, 1978b). With Daphnia magna as the test organism in a 48-hour static experiment, the EC50 for TCI was 85.2 mg/l. In a chronic test with this organism, no adverse effects were found at the highest test concentrations of 10 mg/l.

Investigation of the bioconcentration potential of TCI for bluegill indicated that the bioconcentration factor (BCF) was 17. The half-life of this compound in tissues was less than 1 day (EPA, 1980; EPA, 1978b). These data suggested the unlikelihood of residues at exposure concentrations below those directly toxic to aquatic life (EPA, 1980).

Few data are available relating to effects of TCI on saltwater aquatic life. Borthwick (1977) exposed sheepshead minnows and grass shrimp to 20 and 2 mg/l, respectively, and noted erratic swimming, uncontrolled movement, and loss of equilibrium after several minutes.

3.5 CRITERIA, STANDARDS, AND REGULATIONS

The current occupational standard for TCI established by the Occupational Safety and Health Administration in 1972 (General Industry Standards, OSHA, 1978) is 100 ppm (525 mg/m^3) as a time-weighted average (TWA) over an 8-hr period. More recently, the National Institute for Occupational Safety and Health (Page and Arthur, 1978) has noted that the current standard appears to be inadequate for protection of workers from the carcinogenic potential of TCI. A level of 25 ppm, as a TWA, was the recommended standard proposed by NIOSH based upon engineering control capabilities.

The available data for trichloroethylene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 45,000 μg/l, and acute toxicity to saltwater aquatic life occurs at concentrations as low as 2,000 μg/l. Toxic effects would occur at lower concentrations among species more sensitive than those tested (EPA, 1980).

The U.S. Environmental Protection Agency (Ambient Water Quality Criteria Document, 1980) recommended criteria for protection of human health from the potential carcinogenic effects of trichloroethylene exposure from ingestion of contaminated water and aquatic organisms. The levels predicted to result in one additional case of cancer for every 10^5, 10^6, and 10^7 people exposed are 27 μg/l, 2.7 μg/l, and 0.27 μg/l, respectively. Excluding water consumption, the levels are 807 μg/l, 80.7 μg/l, and 8.07 μg/l for consumption of aquatic organisms only. Using a no-observable-effect-level of 2.38 mg/kg/d, a protective ambient water level of 806 μg/l was calculated for protection from other adverse affects (calculated from NCI bioassay, 1976).

In a more recent report (see Appendix) unit risk estimates were calculated both for air and water, based upon continuous lifetime exposure to technical grade TCI. The individual lifetime risk of cancer from an assumed continuous lifetime exposure to 1 μg/m^3 of TCI in air is estimated to be 2.7×10^{-6}; a 95 percent upper-limit estimate of risk is reported to be 1.7×10^{-5}. The corresponding risk for continuous lifetime exposure to 1 μg/l of TCI in water is estimated to be 3.6×10^{-7}.

3.6 RELATIVE SOURCE CONTRIBUTIONS

As previously discussed in this chapter, no trend has clearly been established for the production and consumption of TCI which takes into account erratic fluctuations in imports and exports. Estimated production for 1979 was about 145,000 metric tons. Approximately 80 to 95 percent of the TCI produced in the United States is used for vapor degreasing of fabricated metal parts and emitted to the atmosphere, where it has an estimated residence time of 2 weeks. Concentrations of 32 ppb have been measured for some large urban centers in the United States.

Contamination of water with TCI can occur directly (e.g., effluent from production or user sites) or indirectly from rainout. In addition, TCI can be formed during the chlorination of water (U.S. EPA, 1979). TCI decomposes slowly in dilute aqueous solution, but can evaporate rapidly if the solution is agitated (Section 3.1). In the United States, TCI has been detected in a number of surface and subsurface waters at levels ranging up to 403 ppb and in some finished drinking waters at levels of up to 32 ppb.

Few data concerning the contamination of U.S. foodstuffs with TCI are available. According to a Draft Exposure Assessment for TSPC Solvents (U.S. EPA, 1981b) average levels of TCI in dairy products, meat, fish, eggs, fruits, and grains ranged from 3 to 100 μg per liter or kg and average levels of TCI in potatoes, beverages, oils, and fats ranged from 0 to 20 μg per liter or kg.

Table 3-10, taken from a Draft Exposure Assessment (July) for TSPC Solvents (U.S. EPA, 1981b), presents estimates of relative exposure to TCI from inhalation and ingestion and from dermal absorption. These estimates indicate that, in the absence of occupational exposure, ingestion of contaminated drinking water may constitute a major contribution to human intake of TCI. Dermal absorption of TCI from contaminated water would appear to add little to human intake, but ingestion of contaminated food and inhalation of ambient air can add significantly to the intake of TCI.

TABLE 3-10. INDICATIONS OF RELATIVE EXPOSURE FOR TRICHLOROETHYLENE[a]
(U.S. EPA, July 1981b)

Route of Exposure	Range of Exposures mg/kg/year	Range of Exposures mg/kg/day
I. Inhalation[b]		
A. Occupational	5.3-66,484	.02-255.7
B. Consumer	unknown	unknown
C. Ambient air	.00104->2.61	.0000003->.007
II. Ingestion		
A. Food	.032-.499	.000088-.0014
B. Drinking water	<.0365-3.65	<.0001-.1
III. Dermal absorption		
A. Ambient water	.0000030-.000025	8.2×10^{-9}-6.8×10^{-8}
B. Potable water	0-.0012	0-3.3×10^{-6}

[a] Draft estimates, subject to change

[b] Based on a 20 m^3/day breathing rate; not corrected for absorption

3.7 REFERENCES

Alexander, H. C., W. M. McCarty, and E. A. Bartlett. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bull. Environ. Contam. Toxicol. 20:344-352, 1978.

Altshuller, A. P. Lifetimes of organic molecules in the troposphere and lower stratosphere. Environ. Sci. Technol., 1980 (in press).

Battelle Columbus Laboratories. Environmental Monitoring Near Industrial Sites: Trichloroethylene. Prepared for the U.S. Environmental Protection Agency, PB 273203, August 1977.

Beckers, N. L. Stabilization of Chlorinated Hydrocarbons. Patent No. 3,682,830, U.S. Patent Office, Washington, DC, August 8, 1972.

Bellar, T. A., W. L. Budde, and J. W. Eichelberger. The Identification and Measurement of Volatile Organic Compounds in Aqueous Environmental Samples. Chapter 4. In: Monitoring Toxic Substances. D. Schvetzle, ed., ACS Symposium Series 94, American Chemical Society, 1979.

Bellar, T. A., J. J. Lichtenberg, and R. C. Kroner. The occurrence of organohalides in chlorinated drinking waters. J. Am. Water Works Assoc. 66: 703-706, 1974.

Bonse, G., and D. Henschler. Chemical reactivity, biotransformation, and toxicity of polychlorinated aliphatic compounds. CRC Crit. Rev. Toxicol. 4:395-409, 1976.

Borthwick, P. W. Results of toxicity tests with fishes and macroinvertebrates. Data sheets available from U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL, 1977.

British Department of the Environment. Chlorofluorocarbons and Their Effect on Stratospheric Ozone (Second Report). Pollution Paper No. 15, Her Majesty's Stationery Office, 1979.

Bucon, H. W., J. F. Macko, and H. J. Taback. Volatile Organic Compound (VOC) Species Data Manual. EPA-450/3-78-119. Report prepared for the U.S. Environmental Protection Agency, December 1978.

Campbell, M. J., J. C. Sheppard, and B. F. Au. Measurement of hydroxyl radical concentrations in boundary layer air by monitoring CO oxidations. Geophy. Res. Lett. 6:175-178, 1979.

Chemical and Engineering News, 19 May 1975. pp. 41-43.

Chemical Marketing Reporter. Chemical Profile for Trichloroethylene, 26 June 1978.

Coleman, W. E., R. D. Lingg, R. G. Melton, and F. C. Kopfler. The Occurrence of Volatile Organics in Five Drinking Water Supplies Using Gas Chromatography/Mass Spectrometry. Chapter 21. In: Identification and Analysis of Organic Pollutants in Water. L. H. Keith, ed., Ann Arbor Science, 1976.

Committee on Methods for Toxicity Tests with Aquatic Organisms: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecol. Res. Series, EPA-600/3-75-009, 1975.

Correia, Y., G. J. Martens, F. H. van Mensch, and B. P. Whim. The occurrence of trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane in Western Europe in air and water. Atmos. Environ. 11:1113-1116, 1977.

Cronn, D. R., and D. E. Harsch. Determination of atmospheric halocarbon concentrations by gas chromatography-mass spectrometry. Anal. Lett. 12(B14): 1489-1496, 1979.

Cronn, D. R., and E. Robinson. Determination of Trace Gases in Learjet and V-2 Whole-air Samples Collected During the Intertropical Convergence Zone Study. Report submitted to NASA, Washington State University, August 1978.

Cronn, D. R., R. A. Rasmussen, and E. Robinson. Report for Phase I. Report prepared for U.S.E.P.A., Contract No. R0804033-01, Washington State University, 23 August 1976.

Cronn, D. R., R. A. Rasmussen, E. Robinson, and D. E. Harsch. Halogenated compound identification and measurement in the troposphere and low stratosphere. J. Geophy. Res. 82:5935-5943, 1977a.

Cronn, D. R., R. A. Rasmussen, and E. Robinson. Measurement of Tropospheric Halocarbons by Gas Chromatography/Mass Spectrometry. Report for Phase II of EPA Grant No. R0804033-02, October 1977b.

Crutzen, P., and J. Fishman. Average concentrations of OH in the troposphere and the budgets of CH_4, CO, H_2 and Ch_3CCl_3. Geophy. Res. Lett. 4:321-324, 1977.

Crutzen, P. A., I. S. A. Isaksen, and J. R. McAfee. The impact of the chlorocarbon industry on the ozone layer. J. Geophy. Res. 83:345-362, 1978.

Cundy, J. M. Trichloroethylene in anesthetic practice. Anesthesia 31:950 (Letter), 1976.

Dahlberg, J. A. The non-sensitized photooxidation of trichloroethylene in air. Acta Chem. Scand. 23:3081-3090, 1969.

Derwent, R. G., and A. E. J. Eggleton. Halocarbon lifetimes and concentration distributions calculated using a two-dimensional tropospheric model. Atmos. Environ. 12:1261-1269, 1978.

Dietz, E. A., and K. F. Singley. Determination of chlorinated hydrocarbons in water by headspace gas chromatography. Anal. Chem. 51:1809-1814, 1979.

Dilling, W. L. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11:405-409, 1977.

Dilling, W. L., C. J. Bredeweg, and N. B. Tefertiller. Simulated atmospheric photodecomposition rates of methylene chloride, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other compounds. Environ. Sci. Technol. 10:351-356, 1976.

Dilling, W. L., N. B. Tefertiller, and G. J. Kallos. Evaporation rates and reactivities of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other chlorinted compounds in dilute aqueous solutions. Environ. Sci. Technol. 9:833-838, 1975.

Dowty, B. J., D. R. Carlisle, and J. L. Laseter. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environ. Sci. Tech. 9:762-765, 1975.

Ewing, B. B., E. S. K. Chian, J. C. Cook, C. A. Evans, P. K. Hopke, and E. G. Perkins. Monitoring to Detect Previously Unrecognized p Mutants in Surface Waters. EPA-560/6-77-015. Report prepared by the University of Illinois for the U.S. Environmental Protection Agency, July 1977.

Farm Chemicals Handbook, 1976. Cited in: Waters, E. M., H. B. Gerstner, and J. E. Huff. Trichloroethylene. I. An overview. J. Toxicol. Environ. Health 2:671-707, 1977.

Federal Register 42(131):35314-35316, 8 July 1977.

Fuller, B. B. Air pollution assessment of trichloroethylene. MITRE Technical Report No. MTR-7142, 1976.

Gay, B. W., P. L. Hanst, J. J. Bufalini, and R. C. Noonan. Atmospheric oxidation of chlorinated ethylenes. Environ. Sci. Technol. 10:58-67, 1976.

General Industry Standards. OSHA Safety and Health Standards, (29 CFR 1910) United States Department of Labor Occupational Safety and Health Administration OSHA 2206, revised November 7, 1978.

Graedel, T. E., and D. L. Allara. Tropospheric halocarbons: Estimates of atmospheric chemical production. Atmos. Environ. 10:385-388, 1976.

Grimsrud, E. P., and R. A. Rasmussen. Survey and analysis of halocarbons in the atmosphere by gas chromatography-mass spectrometry. Atmos. Environ. 9:1014-1017, 1975.

Hardie, D. W. F. Chlorocarbons and Chlorohydrocarbons: Trichloroethylene. In: Kirk-Othmer's Encyclopedia of Chemical Technology. Vol. 5. Second edition, 1964. pp. 183-195.

Harsch, D. Study of Halocarbon Concentrations in Indoor Environments. Report to U.S. Environmental Protection Agency. Contract No. WA-6-99-2922-J. July 7, 1977.

Harsch, D. E., and D. R. Cronn. Low-pressure sample-transfer technique for analysis of stratospheric air samples. J. Chromat. Sci. 16:363-367, 1978.

Huff, J. E. New evidence on the old problems of trichloroethylene. Ind. Med. Surg. 40:25-33, 1971.

Humphrey, J., and M. McClelland. Cranial-nerve palsies with herpes following general anaesthesia: a report. Brit. Med. J. 1:315-318, 1944.

Jones, G., and G. Scott. U.S. Bureau of Mines--Report of Investigation 3666, Washington, D.C., 1942. Cited in: Kirk-Othmer's Encyclopedia of Chemical Technology. Volume 5. Third Edition, 1979.

Jungclaus, G. A., V. Lopez-Avila, and R. A. Hites. Organic compounds in an industrial wastewater. A case study of their environmental impact. Environ. Sci. Technol. 12:88-96, 1978.

Knoll, J. E., M. A. Smith, and M. Rodney Midget. Evaluation of emission test methods for halogenated hydrocarbons. Vol. I. EPA-600/4-79-025, March 1979.

Kopfler, F. C., R. G. Melton, R. D. Lingg, and W. E. Coleman. In: Identification and Analysis of Organic Pollutants in Water. L. W. Keith, ed., Ann Arbor Science, 1976. pp. 87-104.

Kummert, R., E. Molnar-Kubica, and W. Giger. Trace determination of tetrachloroethylene in natural water by direct aqueous injection, high-pressure liquid chromatography. Anal. Chem. 50:1637-1639, 1978.

Lillian, D., H. B. Singh, A. Appleby, L. Lobban, R. Arnts, R. Gumpert, R. Hague, J. Toomey, J. Kazazis, M. Antell, D. Hansen, and B. Scott. Atmosphere fates of halogenated compounds. Environ. Sci. Technol. 9:1042-1048, 1975.

Lillian, D., H. B. Singh, and A. Appleby. Gas chromatographic analysis of ambient halogenated compounds. J. Air Pollut. Control Assoc. 26:141-143, 1976.

Lovelock, J. F. Atmospheric halocarbons and stratospheric ozone. Nature 252: 292-294, 1974a.

Lovelock, J. F. The electron capture detector: Theory and practice. J. Chromat. 99:3-12, 1974b.

Makide, Y., Y. Kanai, and T. Tominaga. Background atmospheric concentrations of halogenated hydrocarbons in Japan. Bull. Chem. Soc. Japan 53:2681-2682, 1980.

Mapleson, W. W. An electric analogue for uptake and exchange of inert gases and other agents. J. Appl. Physiol. 18:197-204, 1963.

McConnell, G., O. M. Ferguson, and C. R. Pearson. Chlorinated hydrocarbons in the environment. Endeavor 34:13-18, 1975.

McNeill, W. C. Chlorocarbons and Chlorohydrocarbons. Trichloroethylene. In: Kirk-Othmer's Encyclopedia of Chemical Technology. Volume 5. Third Edition, 1979. pp. 745-753.

Metz, L., and A. Roedy. Chem. Ing. Technile. 21:191, 1949. Cited in: Kirk-Othmer's Encyclopedia of Chemical Technology. Volume 5. Third Edition, 1979.

Murray, A. J., and J. P. Riley. Occurrence of some chlorinated aliphatic hydrocarbons in the environment. Nature 242:37-38, 1973a.

Murray, A. J., and J. P. Riley. The determination of chlorinated aliphatic hydrocarbons in air, natural waters, marine organisms, and sediments. Anal. Chim. Acta 65:261-270, 1973b.

National Academy of Science. Ozone and Other Photochemical Oxidants. Committee on Medical and Biologic Effects of Environmental Pollutants, 1977.

National Academy of Science. Nonfluorinated Halomethanes in the Environment. Panel on Low Molecular Weight-Halogenated Hydrocarbons. Coordinating Committee for Scientific and Technical Assessments of Environmental Pollutants, 1978.

National Bureau of Standards. Reaction Rate and Photochemical Data for Atmospheric Chemistry-1977. R. F. Hampson, Jr. and D. Garvin, eds., May 1978.

National Institute of Occupational Safety and Health. Occupational Exposure to Phosgene. HEW Publication No. 76-137, 1976.

Noweir, M., E. A. Pfitzer, and T. F. Hatch. Decomposition of chlorinated hydrocarbons. A review. Am. Ind. Hyg. Assoc. J. 33:454-460, 1973.

Page, N. P., and J. L. Arthur. Special Occupational Hazard Review of Trichloroethylene, NIOSH, January 1978.

Pasquini, D. A. Evaluation of glass sampling tubes for industrial breath analysis. Am. Ind. Hyg. Assoc. J. 39:55-62, 1978.

Pearson, C. R., and G. McConnell. Chlorinated C_1 and C_2 hydrocarbons in the marine environment. Proc. Roy. Soc. London B. 189:305-332, 1975.

Pellizzari, E. D. Electron capture detection in gas chromatography. J. Chromatogr. 98:323-361, 1974.

Pellizzari, E. D. Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors EPA-600/2-75-075, November 1975.

Pellizzari, E. D., J. E. Bunch, R. E. Berkley and J. McRae. Collection and analysis of trace organic vapor pollutants in ambient atmospheres. The performance of Tenax GC cartridge sampler for hazardous vapors. Anal. Lett. 9:45-63, 1976.

Pellizzari, E. D. The Measurement of Carcinogenic Vapors in Ambient Atmospheres. EPA-600/7-77-055, June 1977.

Pellizzari, E. D., M. D. Erickson, and R. A. Zweidinger. Formulation of a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media. EPA-560/13-79-006, U.S. Environmental Protection Agency, July 1979.

Piet, G. J., P. Slingerland, F. E. deGrunt, M. P. M. V. d. Heuvel, and B. C. J. Zoeteman. Determination of very volatile halogenated organic compounds in water by means of direct headspace analysis. Anal. Lett. A11:437-448, 1978.

Powell, J. F. Solubility or distribution coefficient of trichloroethylene in water, whole blood, and plasma. Brit. J. Indust. Med. 4:233-236, 1947.

Rasmussen, R. A., D. E. Harsch, P. H. Sweany, J. P. Krasnec, and D. R. Cronn. Determination of atmospheric halocarbons by a temperature-programmed gas chromatographic freezeout concentration method. J. Air Pollut. Control Assoc. 27:579-581, 1977.

Renberg, L. Determination of volatile halogenated hydrocarbons in water with XAD-4 resin. Anal. Chem. 50:1836-1838, 1978.

Russell, J. W., and L. A. Shadoff. The sampling and determination of holocarbons in ambient air using concentration on porous polymer. J. Chromatogr. 134: 275-284, 1977.

Sato, A., and T. Nakajima. A structure-activity relationship of chlorinated hydrocarbons. Arch. Environ. Health 34:69-75, 1979.

Scherb, K. Studies on the evaporation of several low-molecular weight chlorinated hydrocarbons from a flowing channel. Muench eeitr. abwasser-fischflussboil. 30:235-248, 1978.

Schlossberg, L. Letter to Paul Price, U.S. Environmental Protection Agency, Washington, DC, June 26, 1980.

Singh, H. B. Phosgene in the ambient air. Nature 264:428-429, 1976.

Singh, H. B. Atmospheric halocarbons: Evidence in favor of reduced average hydroxyl radical concentration in the troposphere. Geophy. Res. Lett. 4:101-104, 1977.

Singh, H. B. Discussions concerning trichloroethylene. Atmos. Environ. 12:1809, 1978.

Singh, H. B., L. Salas, D. Lillian, R. R. Arnts, and A. Appleby. Generation of accurate halocarbon primary standards with permeation tubes. Environ. Sci. Technol. 11:511-513, 1977.

Singh, H. B., L. J. Salas, H. Shigeishi, A. J. Smith, E. Scribner, and L. A. Cavanagh. Atmospheric Distributions, Sources, and Sinks of Selected Halocarbons, Hydrocarbons, SF_6, and N_2O. EPA-600/3-79-107, U.S. Environmental Protection Agency, November 1979.

Singh, H. B., L. J. Salas, A. J. Smith, and H. Shigeishi. Measurements of some potentially hazardous organic chemicals in urban environments. Atmos. Environ. 15:601-612, 1981.

Sjöberg, B. Thermal decomposition of chlorinated hydrocarbons. Svensk. Kem. Tid. 64:63-79, 1952.

Smith, J. H., D. C. Bomberger, Jr., and D. L. Haynes. Prediction of the volatilization rates of high-volatility chemicals from natural water bodies. Environ. Sci. Technol. 14:1332-1337, 1980.

SRI International. Directory of Chemical Producers, 1978.

SRI International. In: Chemical Economics Handbook. Current Indicator Supplemental Data 632.3001J; 632.3001V; 632.3001W, Menlo Park, CA, 1981.

Tada, T., T. Ohta, and I. Mizoguchi. Behavior of chlorinated hydrocarbons in urban air. Ann. Rep. Tokyo Metr. Res. Lab. P.H. 27:242-246, 1976.

U.S. Environmental Protection Agency. Preliminary Study of Selected Potential Environmental Contaminants - Optical Brighteners, Methyl Chloroform, Trichloroethylene, Tetrachloroethylene, Ion Exchange Resins. EPA-560/2-75-002, July 1975.

U.S. Environmental Protection Agency. Air Quality Criteria for Ozone and Other Photochemical Oxidants. EPA-600/8-78-004, April 1978a. pp. 5-52.

U.S. Environmental Protection Agency. In-Depth Studies on Health and Environmental Impacts of Selected Water Pollutants. Contract No. 68-01-4646, Duluth, MN, 1978b.

U. S. Environmental Protection Agency. An Assessment of the Need for Limitations on Trichloroethylene, Methyl Chloroform, and Perchloroethylene. EPA-560/11-79-009, July 1979.

U.S. Environmental Protection Agency. Ambient Water Quality Criteria: Trichloroethylene. EPA-440/5-80-077, October 1980.

U.S. Environmental Protection Agency. Exposure Assessment: The Occurrence of Volatile Organic Chemicals in Drinking Water. Office of Drinking Water, Washington, DC, March 1981a.

U.S. Environmental Protection Agency. Draft Exposure Assessment for TSPC Solvents. TSPC Solvents Work Group No. 2, July 1981b.

U.S. International Trade Commission. Synthetic Organic Chemicals. United States Production and Sales. USITC Publication 920, 1978.

Waters, E. M., H. B. Gerstner, and J. E. Huff. Trichloroethylene. I. An overview. J. Toxicol. Environ. Health 2:671-707, 1977.

Zoeteman, B. C. J., K. Harmsen, J. B. H. J. Linders, C. F. H. Morra, and W. Slooff. Persistent organic pollutants in river water and ground water of the Netherlands. Chemosphere 9:231-249, 1980.

4. MAMMALIAN DISPOSITION AND FATE

4.1 INTRODUCTION

Trichloroethylene (TCI, 1,1,2-trichloroethylene), a chloroalkene, is a highly volatile liquid whose vapors exert pharmacologic effects common to general inhalation anesthetics. Although it is no longer widely used as an anesthetic or analgesic agent, the highly effective fat-solvent properties of TCI are the basis of its numerous industrial uses and its use as an extractant in the food processing industry (Chapter 3.1). At work sites where this compound is manufactured or used, the principal mode of entry into the body is by inhalation and lung absorption of TCI vapor. Oral and dermal absorption are secondary routes of absorption. Trichloroethylene is present in the water supplies of many of our cities and in their atmospheres and it has been identified in measurable amounts in the food chain (McConnell et al., 1975; Dowty et al., 1975; NIOSH Survey, 1977; OSHA, 1978; Chapter 3.3.3). Hence, ordinary exposure in the non-industrial environment includes air, food and drinking water. Indeed, TCI has been detected in notable amounts in the breath of healthy humans and in postmortem human tissue samples (Conkle et al., 1975; McConnell et al., 1975).

This chapter reviews the available literature on the absorption, tissue distribution, metabolism and excretion of TCI in mammalian organisms with particular attention to man. These factors underlie the health effects of exposure to TCI (Chapters 5, 6) and its mutagenic and carcinogenic potential (Chapter 7). In addition, measures for exposure and body burden, the relation of toxicity to metabolism, and interactions with other xenobiotics are also considered.

Because of its wide use as an industrial solvent since the First World War and as a volatile general anesthetic for forty years (now of only limited use in the USA, but in wide use in Europe), most of the available information concerns the absorption and disposition of TCI after inhalation. Studies have been reported of both single and repeated experimental exposures to 100 ppm or less (current TLV), and also to air concentrations relevant to its medical use as an analgesic (0.5 vol. percent; 5000 ppm) and as an anesthetic (0.5 to 1 vol. percent; 10,000 ppm). Because of its low vapor pressure, a saturated vapor solution of TCI at 25°C and 760 mmHg contains only about 10 vol. percent TCI (Hall et al., 1953). The advent in recent years of gas chromatographic methods for measuring TCI and its metabolites (Monster and Boersma, 1975; Ertle et al., 1972; Muller et al., 1972; Kimmerle and Eben, 1973a,b; Breimer et al., 1974) has greatly advanced earlier studies utilizing the Fujiwara color reaction method (1914) and its modifications (Seto and Schultze, 1956; Tanaka and Ikeda, 1968). It is to be hoped that these more specific and sensitive analytical methods will stimulate quantitative studies on oral and dermal absorption for which very little information is presently available.

4.2 ABSORPTION AND DISTRIBUTION

4.2.1 Oral

The kinetics of gastrointestinal absorption of TCI after oral ingestion have not been specifically studied. However, TCI is an uncharged, nonpolar and highly lipophilic compound (Table 4-1) and consequently can be expected to readily cross the gastrointestinal mucosal barrier by passive diffusion. Daniel (1963) dosed rats by stomach-tube with 36 Cl-labeled TCI liquid (40

TABLE 4-1. PARTITION COEFFICIENTS OF TCI FOR VARIOUS BODY TISSUES AND COMPONENTS OF RAT AND MAN

Rat	Mean Values
Blood/air	25.82
Lung/blood	1.03
Heart/blood	1.10
Kidney/blood	1.55
Liver/blood	1.69
Muscle/blood	0.63
Brain/blood	1.29
Testes/blood	0.71
Spleen/blood	1.15
Fat/blood	25.59
Man	
Blood/air	9.92
Fat/air	674.4
Lethicin/air	387.9
Chloesterol/air	52.15
Cholesterol oleate/air	261.93
Triolein/air	848.24

Adapted from Sato et al., 1977.

to 60 mg/kg b.w.) and recovered 90-95% of the label in expired air and urine, suggesting virtually complete absorption by this route. In man, absorption through the gastrointestinal mucosa is also extensive, as documented by the numerous cases of poisoning by oral ingestion reported over the years (Defalque, 1961; Vyskocil and Polak, 1963; Wiecko, 1966; Vignoli et al., 1970; Midgal et al., 1971). Perhaps because of its sweet smell and the elation it produces, many incidences of vapor addiction, as well as reports of ingestion as a substitute for ethanol (Defalque, 1961) and abuse by "solvent sniffers" (Smith, 1966; Baerg and Kimberg, 1970), have been recorded.

4.2.2 Dermal

Skin absorption from TCI vapor exposure is usually negligible, although direct skin contact with the liquid by immersion of the hands, or partial body immersion, may result in absorption. Uptake through the skin is related to the type of skin exposed and the area of skin exposure as well as to the duration of skin exposure. Tsuruta (1978) estimated skin absorption of TCI in mice by *in vivo* and *in vitro* techniques. The percutaneous absorption rate of TCI in mice was reported to be between 59.8 and 92.4 $\mu mol/min/cm^2$ or between 7.82 and 12.1 $\mu g/min/cm^2$. Assuming that the approximate surface area of both hands (800 cm^2) is immersed in TCI for 1 min and that TCI is absorbed at the rate of 7.82 to 12.1 $\mu g/min/cm^2$, the quantity of TCI absorbed through the skin is estimated by this investigator to be 6.26 to 9.68 mg. Tsuruta claims this value suggests that direct contact with TCI, even if the percutaneous absorption rate of TCI is different between human skin and mouse skin, may have the potential to contribute importantly to the overall exposure to TCI. However, Stewart and Dodd (1964) have experimentally estimated skin absorption of TCI in volunteers by noting its appearance and concentration in lung alveolar air after thumb immersion. The mean peak concentration of TCI in expired air 30 minutes after immersion was only 0.5 ppm, indicating that the rate of absorption through the skin is very slow, even after allowing for the possibility of lipid storage and for metabolism to urinary metabolites (vide infra). In addition, Sato and Nakajima (1978) demonstrated that, in skin exposure in man, an appreciably large proportion of absorbed TCI is eliminated unchanged through the lungs. These authors also concluded that TCI would rarely be absorbed through the human skin in toxic amounts during normal industrial use.

4.2.3 Pulmonary Absorption and Tissue Distribution

Inhaled TCI rapidly equilibrates across the lung alveolar endothelium. The blood/gas partition coefficient of 9.92 at 37°C is comparable to the anesthetic gases--chloroform, diethylether, and methoxyfluorane--while its lipid solubility is considerably higher (olive oil/gas coefficient, 960 at 37°C) (Powell, 1947; Eger and Larson, 1964). Thus, pulmonary uptake of TCI is high with considerable storage in lipid body tissues. TCI distributes to all body tissues and appears in sweat and saliva (Bartonicek, 1962). It crosses the blood-brain barrier (anesthetic effect) and the placental barrier with ease. Helliwell and Hutton (1950), investigating the distribution in pregnant sheep and goats, found that TCI appeared in the fetal circulation within 2 min of exposure; at equilibrium the fetal/maternal concentration ratio was unity. The partition coefficients for various tissues of rat and man are given in Table 4-1 (from Sato et al., 1977).

Pulmonary TCI uptake is proportional to the magnitude of the gas partial pressure difference between alveoli and pulmonary venous blood. This difference diminishes when all body tissues achieve equilibrium with the alveolar (arterial) partial pressure. Initially uptake is rapid, but about three hours of exposure are required for complete tissue equilibrium to be achieved (Fernandez et al., 1977; Sato et al., 1977; Monster et al., 1976, 1979; Muller et al., 1974). Thus, at equilibrium with a given concentration of TCI in inspired air, the difference between the inspired air concentration of TCI and alveolar concentration of TCI provides a measure of gas uptake. With appropriate accounting for ventilation (about 4-8 l/min at rest) and duration of exposure, the amount taken up by the body or retention (dose; body burden) can be calculated. At alveolar and tissue equilibrium, the retention reflects disposition within the body by tissue storage, metabolism, or excretion by other routes than the lungs. A retention of 36 percent to 75 percent has been reported for TCI by various investigators (Soucek and Vlachova, 1960; Bartonicek, 1962; Nomiyama and Nomiyama, 1971, 1974a,b), with higher values (70 to 75 percent) reported from more recent studies (Fernandez et al., 1975; Monster et al., 1976, 1979). The retention value is independent of the inspired TCI concentration. In contrast, the amount (dose) of TCI absorbed into the body is directly proportional to the TCI concentration in the inspired air (Fernandez et al., 1975; Monster et al., 1976, 1979; Muller, et al., 1974). The body burden of TCI also increases with duration of exposure, with repeated daily exposures, and with exercise (increased ventilation), at a given inhaled air concentration (Astrand and Ovrum, 1976; Monster et al., 1976, 1979).

The blood concentration of TCI during inhalation and in the elimination phase after exposure closely parallels alveolar gas concentration (Muller et al., 1974; Monster, 1976, 1979; Stewart et al., 1974; Sato et al., 1977; Fernandez et al., 1977). The kinetics of tissue uptake, accumulation and equilibration of TCI is a function of blood concentration, and also of the blood flow/tissue mass and the relative solubilities of TCI in tissues (Table 4-1). Therefore, TCI most rapidly attains equilibrium by passive diffusion into the vessel-rich group of tissues (VRG) (brain, heart, kidneys, liver, endocrine and digestive systems), more slowly with the lean mass (MG) (muscle and skin), and lastly with adipose tissue (FG). As determined from elimination kinetics following exposure, TCI distributes from blood into these three major compartments at approximate rate constants of VRG 17 hr^{-1} (t 1/2, 2.4 min), MG 1.7 hr^{-1} (t 1/2, 25 min), and FG 0.2 hr^{-1} (t 1/2, 3.4 hr) (Fernandez et al., 1975, 1977; Sato et al., 1977). While the lean muscle mass is 50 percent of the body volume versus 20 percent for adipose tissue, saturation and desaturation proceeds more rapidly from the MG compartment (t 1/2, 25 min) than from the FG compartment (t 1/2, 3.4 hr) because of the considerably greater solubility of TCI in lipid (Table 4-1). Inter-person variations in TCI uptake (dose) are therefore influenced primarily by lean body mass and secondarily by adipose tissue mass (Monster et al., 1976, 1979).

4.2.4 Pharmacokinetics of TCI Disposition

Withey and Collins (1980) studied the pharmacokinetics of TCI disposition in the rat after intravenous injection. This mode of administration facilitates the study of distribution and elimination because it bypasses absorption. The time course of TCI concentrations in blood was followed after administration of doses ranging from 3.0 to 15.0 mg/kg body weight. With increasing doses, the data indicated a shift from a two compartment to a three compartment model. Because no evidence of Michaelis-Menton kinetics was observed, this dose range was thought to be below the level that would have saturated rate-limiting metabolic pathways. Half-lives for the terminal phase of elimination from blood ranged from 18 minutes for the low dose to 57 minutes at the high dose, indicating fairly rapid elimination due to metabolism or excretion.

Elimination of TCI from the tissues was studied only at the highest dose because detection limits precluded obtaining data at the lower doses. The rates of elimination of TCI from most tissues (heart, liver, lung, kidney, brain, spleen) were similar to those from blood, indicating that the major organs plus the blood constituted the central pharmacokinetic compartment. No uptake phase was observed. In contrast, the time course of TCI concentrations in perirenal fat indicated an uptake and an elimination phase consistent with a one-compartment pharmacokinetic model. Elimination of TCI from fat was considerably slower ($t_{1/2}$ = 215 min) than elimination from other organs. This half-life for elimination from fat is similar to the estimate (given in Section 4.2.3) of TCI desaturation from adipose tissue ($t_{1/2}$ = 3.4 hours) after inhalation exposure.

4.3 EXCRETION

The total elimination of absorbed TCI involves two major processes, pulmonary excretion of unchanged TCI and hepatic biotransformation to urinary metabolites. The details of hepatic biotransformation of TCI are reviewed below. In man, the principal end metabolites are trichloroethanol (TCE), TCE-glucuronide, and trichloroacetic acid (TCA). The principal, but probably not sole, route of excretion of these metabolites is renal.

4.3.1 Pulmonary Elimination

Pulmonary elimination of TCI after exposure occurs nearly as an inverted reproduction of its uptake. Alveolar concentration parallels blood concentration; both follow exponential concentration decay curves showing three major components of approximate half-times of 2 to 3 min, 30 min, and 3.5 to 5 hr (Fernandez et al., 1977; Sato et al., 1977; Muller, 1974; Nomiyama and Nomiyama, 1971; Monster et al., 1979), represented by VRG, MG and FG compartments, respectively. The long half-time of elimination from adipose tissue (3.5 to 5 hr) explains why after a single short exposure (ex. 4 to 6 hrs) exhaled air contains a notable concentration of TCI 18 hr later, and also why accumulation of TCI (body burden) occurs with repeated daily exposures.

Several investigators (Fernandez et al., 1977; Monster et al., 1979) have shown that, for volunteers exposed to 70 to 100 ppm 4 hr daily for 5 days, the concentration of TCI in blood and exhaled air was 2 to 3 times higher 18 hours after the last exposure than at the comparable time after a single exposure. TCI accumulation was highest in those volunteers with a proportionally larger ratio of adipose to lean muscle mass.

4.3.2 Urinary Metabolite Excretion

The urinary metabolites of TCI (total amount, excretion ratios, and excretion time-course) have always been of considerable interest over the years as quantitative indices of exposure and body burden. Recent investigations have determined that the half-time for renal elimination of TCE and of TCE-glucuronide approximates 10 hr (range 7 to 14 hr) after TCI exposure (Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976, 1979). This value is in agreement with that observed after ingestion of TCE itself (Breimer et al., 1974; Muller et al., 1974). As might be predicted from this relatively short half-time of renal elimination, blood concentrations of TCE and TCE-glucuronide after repeated daily TCI exposures reach a "plateau concentration" (with peaks for each daily exposure) that is dependent on the inhaled air concentration of TCI (Monster et al., 1979; Muller et al., 1974; Fernandez et al., 1977). Consequently, the same daily amount of TCE and TCE-glucuronide is excreted into the urine during repeated daily exposures to the same air concentration of TCI.

In contrast to the short half-life of TCE, the half-time of renal elimination of TCA after TCI exposure approximates 52 hr (range 35 to 70 hr) (Soucek and Vlachova, 1960; Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976). Similar values have been observed when TCA is directly administered to men (82 hr, Paykoc and Powell, 1945; 51 hr, Muller et al., 1974). Distribution of TCA in the body is restricted to the extracellular water space (Paykoc and Powell, 1945); it is very tightly and extensively bound to plasma protein and excluded from erythrocytes, so that plasma concentration is twice as high as whole blood (Soucek and Vlachova, 1960; Monster et al., 1979). As a consequence of the long half-life of TCA, and of the constant metabolic formation of TCA from TCE, blood concentrations of TCA smoothly rise during a single TCI exposure, reaching a maximum 24 to 48 hours post exposure before declining exponentially for 10 to 15 days. The blood concentration of TCA during the rising phase and at maximum is proportional to the TCI inspired air concentration and the duration of the exposure, i.e., to the retained TCI dose (Soucek and Vlachova, 1960; Monster et al., 1976; Sato et al., 1977; Fernandez et al., 1977). During repeated daily exposures, TCA accumulates in the blood exponentially, and declines only 24 to 48 hours after the last exposure (Muller et al., 1974; Fernandez et al., 1977; Monster et al., 1979). Consequently the daily amount of TCA excreted into the urine increases during repeated exposure and then decreases slowly over a period of 15 to 20 days. Several investigators have documented an apparent increase in the half-time of renal elimination of TCA (to 100+ hr) with repeated daily exposures to TCI. This phenomenon is explained by continuous formation of TCA from accumulated tissue stores of TCI and TCE, and by the very tight binding of TCA to plasma protein.

The renal elimination kinetics of TCE and of TCA readily explain the urinary excretion-ratio time-course of these metabolites. The ratio of daily urinary metabolites, TCE/TCA (mg/day), is highest (2:1) 24 hours after a single TCI exposure or after repeated daily exposures (Soucek and Vlachova, 1960; Muller et al., 1972, 1974; Fernandez et al., 1975; Nomiyama and Nomiyama, 1971, 1977; Sato et al., 1977; Monster et al., 1976, 1979). Thereafter the relationship of TCE/TCA progressively decreases from day to day and becomes less than unity at about the sixth day following exposure. Several additional factors are known to influence the excretion ratio, TCE/TCA. The ratio is reported to be markedly greater in males than females for the first 24 hr after exposure (Nomiyama and Nomiyama, 1971, 1977), and TCA urinary excretion is reported to vary diurnally (Soucek and Vlachova, 1960; Monster et al., 1979).

4.4 MEASURES OF EXPOSURE AND BODY BURDEN

Trichloroethylene is principally absorbed by the lungs during industrial or ambient air exposure. However, as worker exposure periods and air concentrations are likely to vary, air analysis does not necessarily provide a reliable indication of true exposure. An index of a worker's current exposure and total body burden can be estimated in accordance with the amount of TCI absorbed and retained as predicted by the known pharmacokinetics and metabolism of TCI. Therefore, monitoring TCI levels in blood or expired air and measuring urinary metabolites are the most common methods for determining the body burden (Stewart et al., 1962, 1970, 1974; Morgan et al., 1970; Nomiyama, 1971, Ogata et al., 1971; Ertle et al., 1972; Ikeda et al., 1972; Muller et al., 1972, 1974; Pfaffli and Blackman, 1972; Kimmerle and Eben, 1973a,b; Lowry et al., 1974; Vesterburg and co-workers, 1976; Fernandez et al., 1975, 1977; Ikeda, 1977; Monster et al., 1976, 1979). These methods, however, are subject to high inter-personal variations in the pharmacokinetics and metabolism of TCI that limit their predictive value. Some of the factors now known to contribute to these variabilities are pulmonary dysfunctional states, inter-individual differences in intrinsic liver metabolism capacity for TCI, modification of metabolism by drugs and environmental xenobiotics, age, sex, exercise or work load and body anthropometry. Stewart and his associates (1974) are vigorous proponents of the value of post-exposure alveolar concentrations of TCI as measures of recent exposure and body burden. However, this method is highly dependent on time of sampling and influenced by individual variations in metabolism of TCI. Muller et al. (1974) have concluded that neither TCA excretion nor the sum of urinary metabolites can be taken as a representative parameter of previous exposure, except possibly for 24 hr daily collections. These workers favor either alveolar TCI concentrations or blood analysis of TCA or TCI as the best present indicators of exposure and body burden. Monster et al. (1979) concluded from their studies that the most "promising parameter for biological monitoring in repeated exposure to TCI" is blood TCA concentration level, because of the ease of sampling and GC analysis, the greater independence of time after exposure, accumulation with chronic exposure and increased body burden, and the smaller inter-individual variation of this parameter.

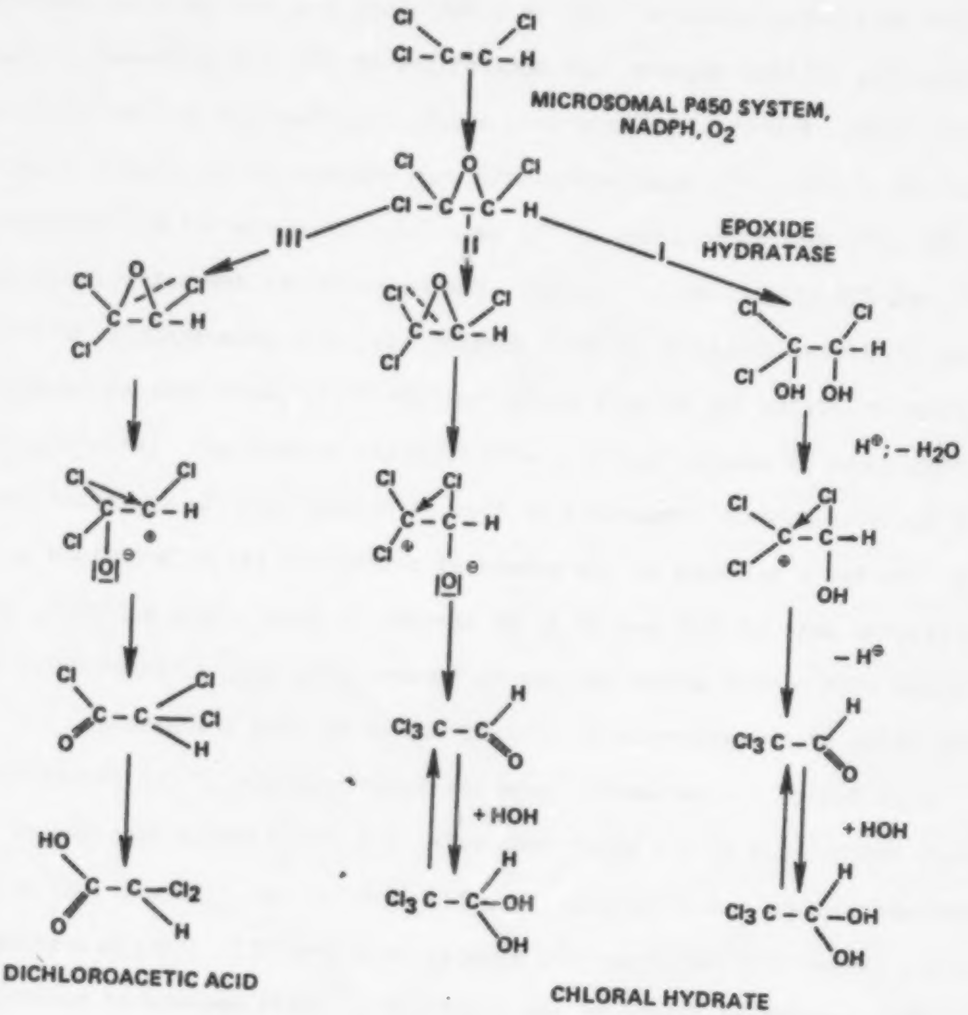
Greater and more precise information than is now available on the absorption, distribution and metabolism kinetics of TCI is required for better means of estimating or predicting body burdens with exposure. To this end, Fernandez et al. (1977) and Sato et al. (1977) have developed mathematical models for studying the pharmacokinetics of inhaled TCI and its metabolites.

4.5 BIOTRANSFORMATION

It has long been known that TCI is appreciably metabolized in the body to TCE and TCA. Most of this metabolism occurs in the liver; however, biotransformation of TCI to these metabolites has also been shown to occur in the lungs (Dalbey and Bingham, 1978), and possibly occurs to a slight extent in the kidneys. Following the introduction in the 1930's of TCI as a general anesthetic, Barrett and co-workers (1939) isolated TCA from the urine of dogs and human patients anesthetized with TCI. Powell (1945) demonstrated TCA in both plasma and urine.

She also observed that TCA continued to be excreted for more than 2 days after a single anesthetic exposure. Butler (1949), who had previously discovered that the hypnotic, chloral hydrate, was metabolized to TCE, TCE-glucuronide and TCA (Butler, 1948), hypothesized that this widely used hypnotic was an intermediate metabolite of TCI. His experiments with dogs exposed to TCI clearly demonstrated that the principal metabolites of TCI were TCE, TCE-glucuronide ("urochloralic acid") and TCA (Figure 4-1). However, Butler could not demonstrate chloral hydrate in either plasma or urine. Nonetheless, this demonstration of biotransformation of TCI to TCE as well as TCA was the first proof that an inhalation anesthetic can be metabolized to a with hypnotic properties. Trichloroethanol has a hypnotic potency comparable to that of chloral hydrate (Marshall and Owens, 1954). Butler's estimate of the extent of metabolism (as a percentage of the administered dose of TCI) was 60 to 80 percent in dogs. This estimate, which is in accord with recent determinations in humans (vide supra), underscores the early appreciation of the extensive biotransformation of this haloalkene.

Since Butler's experiments, numerous investigations of the plasma and urinary metabolites of TCI after industrial and experimental exposure of humans and various animal species have been carried out. In all available reports, the metabolites found and studied have been TCE, TCE-glucuronide and TCA. Only one report exists of the excretion of small amounts of monochloroacetic acid by man after TCI inhalation (Soucek and Vlachova, 1960). This metabolite (4 percent of the TCI administered) was found in urine a few minutes after onset of TCI inhalation, reaching a maximum at the end of exposure with an exponential excretion thereafter. No other end products of TCI metabolism in man have been convincingly demonstrated. Chloroform and dichloroacetic acid have frequently been suggested as metabolites, particularly in the early


MICROSOMAL P450 SYSTEM, NADPH, O2
EPOXIDE HYDRATASE
III
II
I
H⁺; − H2O
− H⁺
+ HOH
+ HOH
DICHLOROACETIC ACID
CHLORAL HYDRATE

INTERACTIONS OF UNSTABLE EPOXIDE AND OF CARBONIUM IONS:

- COVALENT BINDING TO CELLULAR MACRO MOLECULES
- CONJUGATION WITH REDUCED GLUTATHIONE
- ACYL CHLORIDE REACTIONS WITH CELLULAR COMPONENTS [OH, SH, NH_2]

Figure 4-1. Trichloroethylene epoxide formation and rearrangements. (Based on studies cited in text.

literature, but there is no evidence of biotransformation to these metabolites in man (Defalque, 1961; Monster et al., 1976, 1979). Therefore, it is now generally assumed that only TCA and TCE and its glucuronide are the end metabolites to be considered in determining the enzymatic pathway of TCI metabolism (see Figure 4-1). The intermediate metabolite, chloral hydrate, has now been found in the plasma of rats (Kimmerle and Eben, 1973a) and humans inhaling TCI (Cole et al., 1975), and has been shown to be produced in the isolated rat liver upon perfusion with TCI (Bonse et al., 1975), as well as with rat liver microsomal preparations (Byington and Leibman, 1965).

Recent reports agree that "our knowledge of the metabolic pathways of TCI in the organism and the enzyme systems involved is almost complete" (Muller et al., 1975). However, balance studies of TCI in man account for only 70 to 80 percent of the TCI dose (*supra vide*). As in the case of other haloalkenes and alkanes, the possible identification of presently unknown minor metabolites of secondary pathways appears real and assumes some importance with the growing realization that the suspected toxic, mutagenic, teratogenic and carcinogenic potentials of TCI are related to its metabolites (Chapters 6,7).

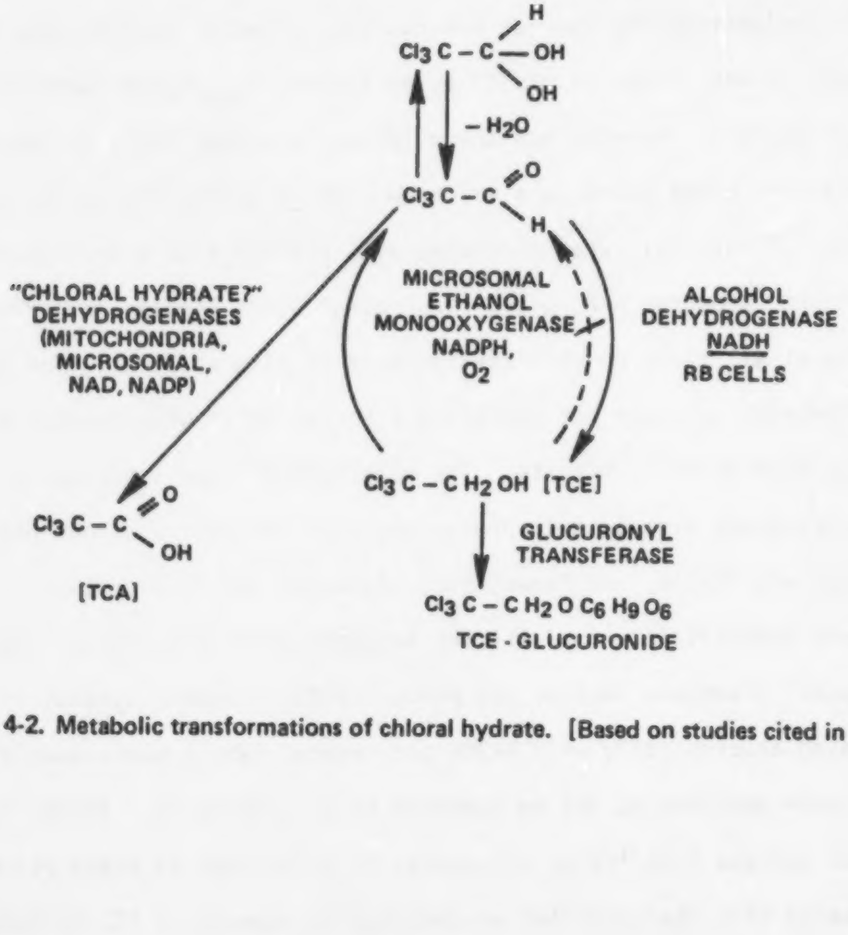
A case in point is the interesting observation by Traylor et al. (1977), from *in vitro* liver microsomal studies, that TCI may undergo a limited conversion to carbon monoxide via highly reactive intermediate metabolites. This conversion may represent a significant minor pathway of TCI biotransformation that has not yet been evaluated. Also, chloroform has been reported as a metabolite of TCI, particularly in the early literature, but no clear evidence of biotransformation to this metabolite in man has been found (Friedman and Cooper, 1960; Defalque, 1961; Monster et al., 1976, and 1979). Pfaffenburger et al. (1980) has reported that TCI is metabolized to chloroform in rats and suggests that chloroform could be a metabolite of trichloroacetic acid in both rats and man.

4.5.1 Enzymatic Pathways of Biotransformation

Current knowledge of the details of the mammalian biotransformation of TCI derives mainly from in vitro studies, principally with liver cell fractions. Figures 4-1 and 4-2 summarize the probable enzymatic pathways involved in the metabolism of TCI as determined from these studies. Among the halogenated hydrocarbons, TCI biotransformation appears to be unusual, since there is neither dehalogenation nor removal of carbon atoms. The initial biotransformation of TCI involves metabolism to chloral hydrate via formation of trichloroethylene epoxide (Figure 4-1). Intramolecular rearrangement with migration of a chlorine atom is presumed to occur in order to explain the genesis of chloral hydrate, TCE and TCA. Conversion of chloral hydrate to trichloroethanol (TCE) and trichloroacetic acid (TCA) is shown in Figure 4-2.

4.5.2 Trichloroethylene Expoxide Formation and Covalent Binding

Powell (1945) suggested many years ago that TCI is probably metabolized to an epoxide, 1,1,2-trichloroethane oxide. Subsequently, Byington and Leibman (1965) conclusively identified chloral hydrate as the product of TCI when incubated with rat liver microsomes fortified with NADPH and O_2 (P_{450} monooxygenase system), thereby verifying the hypothetical chloral hydrate formation proposed by Butler nearly 20 years earlier. These workers also postulated an intermediate formation of an epoxide, analogous to the known microsomal oxidation to epoxides of dihydronapthalene, aldrin, heptachlor and isodrin. Figure 4-1 illustrates the major rearrangements TCI epoxide may undergo to the formation of chloral hydrate assuming a migration of a chlorine atom. Daniel (1963) provided evidence that chloride migration must occur since no dilution of ^{36}chlorine atoms with chlorine atoms from other body pools was observed in the conversion of ^{36}Cl-TCI to its end products TCE and TCA. Bonse et al. (1975) synthesized TCI oxide and found thermal rearrangement occurred without



$Cl_3C-C(H)(OH)(OH)$
$-H_2O$
$Cl_3C-C(=O)H$
"CHLORAL HYDRATE?" DEHYDROGENASES (MITOCHONDRIA, MICROSOMAL, NAD, NADP)
MICROSOMAL ETHANOL MONOOXYGENASE NADPH, O_2
ALCOHOL DEHYDROGENASE NADH RB CELLS
$Cl_3C-C(=O)OH$
[TCA]
Cl_3C-CH_2OH [TCE]
GLUCURONYL TRANSFERASE
$Cl_3C-CH_2OC_6H_9O_6$
TCE - GLUCURONIDE

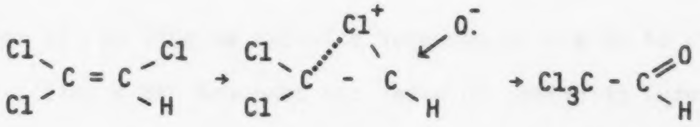
Figure 4-2. Metabolic transformations of chloral hydrate. [Based on studies cited in text].

a chlorine migration forming both an acyl chloride and dichloroacetic acid in water or methanol in vitro (Figure 4-1, pathway III). However, when they perfused TCI from the isolated perfused rat liver only chloral hydrate and the secondary metabolites of chloral hydrate, TCE and TCA, were found. Dichloroacetyl chloride or dichloroacetic acid was not found in the perfusion solution. This did not, however, rule out entirely acyl chloride formation from an epoxide, since the acyl chloride or the intermediate carbonium ion (Figure 4-1, pathway III) might be expected to immediately react with tissue components.

Trichloroethylene epoxide has not been directly demonstrated as a transient compound in the oxidation of TCI by microsomal P_{450} mixed function enzymes to chloral hydrate. Nonetheless the evidence is compelling. In addition to the observations cited above, the evidence from in vitro studies can be summarized as follows: First, TCI, when incubated with rat and rabbit microsomes aerobically with NADPH, produces a P_{450} type I spectral shift (Kelley and Brown, 1974; Uehleke et al, 1976a,b) characteristic of certain substrates for cytochrome P_{450}. Trichloroethylene does not exhibit a spectral shift when incubated with microsomes reduced with CO or dithionate. The magnitude of type I binding of TCI is enhanced with microsomes from animals pretreated with phenobarbital but not 3-methylcholanthrene (Kelley and Brown, 1974; Pelkonen and Vainio, 1975). Trichloroethylene competitively inhibits the metabolism of hexobarbital and aniline by microsomal fractions (Kelley and Brown, 1974). Second, synthetic TCI epoxide incubated anaerobically with NADPH and reduced rabbit microsomes exhibits a difference spectrum at 452 nm (Uehleke et al., 1976a,b). Third, formation of chloral hydrate from TCI by microsomes is stimulated in preparations from animals pretreated with phenobarbital or chronically exposed to TCI (Leibman and McAllister, 1967). Urinary metabolites of TCI are also increased after pretreatment of animals with phenobarbital or polychlorinated biphenyls (Moslen et al., 1977a). Fourth, ^{14}C-TCI or ^{14}C-trichloroethylene epoxide, when incubated aerobically with mouse liver microsomes and NADPH, produce "reactive products" which covalently bind to microsomal protein and DNA (Van Duuren, 1977; Uehleke and Poplawski-Tabarelli, 1977; Bolt et al., 1977; Greim et al., 1977). Binding is decreased by SKF-525A, which inhibits P_{450} metabolism, and by an inhibitor of epoxide hydratase, trichloropropane epoxide. Phenobarbital enhances the binding of TCI to microsomal proteins (Van Duuren, 1977). The addition of competing nucleophiles such as reduced glutathione, imidazole or mercaptoethanol decreases the binding of TCI to microsomal protein.

The details of the enzymatic mechanism(s) of conversion of trichloroethylene epoxide to chloral (trichloroacetaldehyde), which is hydrated immediately to chloral hydrate, are unknown. Bonse et al. (1975) and Kline and Van Duuren (1977) have synthesized the epoxide. The compound was found to have a half-life of 25 min in nonpolar solvents at 60°C and to rearrange to form dichloroacetyl chloride; in water the compound had a half-life of only 1.3 min at pH 7.4 and 37°C. In aqueous solutions, spontaneous rearrangement with chloride migration and carbonium ion formation normally yields dichloroacetyl chloride (Figure 4-1, pathway III), but in the presence of Lewis acid catalysts ($AlCl_3$ or BF_3) chloral is formed after chloride migration with carbonium ion formation. Henschler (1977) has proposed that the formation of chloral *in vivo* could therefore be due to catalytic action of the iron of cytochrome P_{450} in the trivalent form at the microsomal site of formation of the epoxide (Figure 4-1, pathway II). An alternative mechanism is shown as pathway I in Figure 4-1. The epoxide is enzymatically transformed to a diol by microsomal hydratase. This enzyme has a broad substrate spectrum (Oesch, 1973; Oesch et al., 1971), although trichloroethylene epoxide has not been specifically shown to be a substrate. Rearrangement of trichloroethylene glycol by an enzyme-catalyzed reaction, analogous to the acid-catalyzed pinacol rearrangement for α glycols via a carbonium ion, could then produce chloral. Although trichloroethylene epoxide has not as yet been tested as a substrate for microsomal epoxide hydratase, support for this pathway is provided by the observation that the epoxide hydratase inhibitor, trichloropropane oxide, enhances TCI covalent binding to microsomal protein in vitro (Van Duuren, 1977; Leibman and Ortiz, 1977).

Van Dyke (1977) and Van Dyke and Chenoweth (1965) have proposed that TCI conversion to chloral involves a chloronium ion intermediate in concert with oxidation, presumably by cytochrome P_{450} system. As illustrated, the mechanism does not require an epoxide intermediate. The oxidative attack forces chloride migration to the adjacent carbon if possible as in the case of TCI; if not, then the chlorine bonded to the carbon which is oxidatively attacked is released (dechlorination).



This mechanism also results in "reactive" intermediates which can covalently bind to cell constituents. However, an enzyme-directed oxidative attack is still required to provide the known metabolite chloral instead of dichloro-derivatives.

Chloronium ion formation was also suggested by Byington and Leibman (1965) as an intermediate in the rearrangement of the epoxide or TCI glycol (Figure 4-1, pathways II and III, respectively) to chloral. Again, such a mechanism must be enzyme-directed, since spontaneous aqueous decomposition of the epoxide and the highly unstable glycol yield a dichloro-derivative, but not chloral (Bonse et al., 1975).

A secondary minor pathway for TCI biotransformation has been suggested by Traylor et al. (1977). These investigators found that TCI incubated with rat hepatic microsomes gave rise to carbon monoxide (CO). The conversion was dependent on the P_{450} monooxygenase system. Thus, microsomes obtained from rats pretreated with phenobarbital or 3,4-benzo(a)pyrene and incubated with TCI exhibited a difference spectra similar to that obtained with CO, with a peak absorbance at 451 nm. Addition of hemoglobin progressively decreased absorbance at 451 (because of carboxyhemoglobin trapping), and increased absorbance at 419 nm. CO formation was also identified by infrared spectroscopy. The K_m for the CO formation (5.9×10^{-4} M) was identical to the K_s value determined for TCI. Furthermore, the reaction was inhibited by the addition of metyrapone or α-naphthoflavone, inhibitors of microsomal hydroxylation reactions. However, the capacity for microsomal formation of CO from TCI appeared to be very small, with about 0.5 nmoles CO produced per minute per gram of microsomal protein for liver microsomes from untreated rats and about 3.0 nmoles CO $\min^{-1}$ (g^{-1}) for phenobarbital treatment. A direct comparison of moles of CO formed per mole of chloral hydrate formed by the microsomes was not part of this study but would have provided an index of the relative importance of this suggested pathway. The mechanism of the reaction is not known, but Traylor et al. (1977) speculate that, following the formation of TCI epoxide and diol (pathway I, Figure 4-1), scission of the carbon-carbon bond of the diol occurs giving two fragments that are thermodynamically unstable and decompose to HCl and CO. Further work is needed to confirm that these observations are not due to metabolism of an impurity or a product of the known spontaneous decomposition of TCI, and to evaluate the extent of metabolism by what presently appears to be a very minor pathway.

4.5.3 Metabolism of Chloral Hydrate

The enzymatic pathways for the biotransformation of chloral hydrate formed from TCI exposure, to the plasma and urinary metabolites TCE, TCE-glucuronide, and TCA have received less attention in recent years than the mechanism of chloral hydrate formation. Current knowledge of the enzymatic pathways of chloral hydrate biotransformation is outlined in Figure 4-2. Chloral hydrate is very rapidly metabolized in vivo with a half-life of only a few minutes (Marshall and Owens, 1954; Breimer et al., 1974; Cole et al., 1975).

A number of investigators have concluded from in vitro studies that chloral hydrate is a substrate for alcohol dehydrogenase purified from horse liver and rat liver cytosol fractions (Butler, 1948, 1949; Marshall and Owens, 1954; Friedman and Cooper, 1960; Sellers et al., 1972b). By this reaction, with NADH as the required coenzyme, chloral hydrate is reduced to TCE (K_m, 2.7×10^{-3}M horse enzyme). The reaction is essentially unidirectional, since conversion back to chloral hydrate has never been found (Sellers et al., 1972b; Owens and Marshall, 1955; Friedman and Cooper, 1960). Human red cells also reduce chloral hydrate to TCE (K_m, 4.0×10^{-4}M) (Sellers et al., 1972b). The efficiency of red cells and their relatively large mass may explain the nearly undetectable levels of chloral hydrate in plasma and urine after exposure to TCI, while TCE is prominent in plasma and urine. TCE-glucuronide present in plasma and urine is accepted as resulting from conjugation by hepatic microsomal glucuronyl transferase.

The origin of TCA as a plasma and urinary metabolite of chloral hydrate is not as clearly defined. The most likely reaction for the oxidation of chloral hydrate to TCA would involve the well known enzyme, acetaldehyde dehydrogenase. However, chloral hydrate has been reported not to be a substrate for human acetaldehyde dehydrogenase (Kraemer and Deitrich, 1968; Blair and Bodley, 1969; Sellers et al., 1972b). Cooper and Friedman (1958) obtained from rabbit liver acetone powder a partially purified form of NAD-dependent "chloral hydrate dehydrogenase" which was substrate-specific for chloral hydrate. Acetaldehyde was not a substrate but rather a markedly effective inhibitor. Chloral dehydrogenase has not been identified in any other species even though the rabbit, in comparison with the rat or man, poorly converts TCI to TCA (Table 4-2). Furthermore, Cooper and Friedman (1958) reported that their enzyme was not inhibited by disulfiram. However, orally administered disulfiram is known to markedly decrease excretion of TCA and TCE after TCI exposure to man (Bartonicek and Teisinger, 1962) and to dogs and rats (Forssman, 1955, cited in Soucek and Vlachova, 1960).

TABLE 4-2. DIFFERENCES IN METABOLISM OF TCI
URINARY EXCRETION METABOLITES (mg)*

Trichloroethylene metabolites	Animal species		
	Human	Rabbit	Rat
TCA	221 ± 111	0.9 ± 0.4	0.21 ± 0.05
TCE	343 ± 111	157 ± 15	2.23 ± 2
TCE/TCA	1.9 ± 0.9	207 ± 75	12.5 ± 14

*Doses i.p.: rabbit 500 mg; rat 10 mg; man 315 ppm air for 160 min.

Adapted from: Nomiyama and Nomiyama (1977)

In contrast to earlier studies on aldehyde dehydrogenase that found most of this enzyme activity in the cytosol (Büttner, 1965), more recent studies show that about 80 percent of total activity resides in the mitochondrial and microsomal cellular fractions. Tottmar et al. (1973) described at least two different aldehyde dehydrogenases (NAD and NADP-dependent) in rat liver, localized respectively in mitochondria and microsomes. Grunett (1973) found the NAD-dependent mitochondrial enzyme to have a broad substrate specificity, but chloral hydrate was not a substrate.

Present evidence suggests that chloral hydrate is oxidized in the liver to TCA. For example, Bonse et al. (1975) showed that TCI perfused through the isolated rat liver led to the appearance of TCA, TCE and chloral in the post perfusate. However, the oxidoreductase enzyme responsible for chloral oxidation to TCA has not been firmly established. It is possible that microsomal hepatic enzymes are implicated; TCA may arise from action of a microsomal dehydrogenase on chloral as a substrate and/or from the oxidation of TCE to chloral via the microsomal ethanol oxidative system (MEOS) in a manner analogous to ethanol (Liebler and DeCarli, 1969). The latter reaction is supported by the observation that TCE forms a difference absorption with rat microsomes (Uehleke et al., 1976a,b). Biotransformation of TCE to TCA by tissues other than liver have not been reported, although this possibility might help to explain the extended excretion of this metabolite after a single exposure to TCI.

4.6 SPECIES DIFFERENCES IN MAGNITUDE OF TCI METABOLISM

Estimates of the extent of metabolism in man, as a percentage of retained TCI, have been made from balance studies, i.e., accounting for a retained dose after vapor exposure by measuring pulmonary elimination of TCI and metabolites excreted into the urine. No balance studies have been reported with isotopically labeled TCI in man. Daniel (1963) dosed rats by stomach-tube with ^{36}Cl-labeled TCI liquid (40 to 60 mg/kg b.w.) and found 80 percent of the dose was eliminated via the lungs with a half-time of elimination of about 5 hr; urinary metabolites TCE, TCE-glucuronide and TCA accounted for only 15 percent of the dose. Man, in contrast, appears to metabolize inhaled TCI more extensively (between 40 to 75 percent of the retained dose) as reported by various investigators over the last three decades (Barrett et al., 1939; Powell, 1945; Forssman and Holmqvist, 1953; Soucek and Vlachova, 1960; Bartonicek, 1962; Ogata et al., 1971; Ertle et al., 1972; Muller et al., 1972, 1974, 1975; Kimmerle and Eben, 1973a,b; Stewart et al., 1974; Fernandez et al., 1975, 1977; Nomiyama and Nomiyama, 1971, 1977; Sato et al., 1977; Monster et al., 1976, 1979). The data of Nomiyama and Nomiyama (1977) (Figure 4-3) and of Ikeda (1977), which correlate urinary metabolite excretion with increasing levels of TCI exposure, indicate that liver capacity for metabolism of TCI is nonlimiting at least up to an exposure of 315 ppm for 3 hr (equivalent to 25 mg TCI/kg B.W.). Furthermore, the alveolar to inspired air concentration ratio (25 percent; retention 75 percent) at exposure equilibrium approximates the cardiac output flow through the liver (25 percent) and suggests that TCI is completely removed from blood by the liver in a single pass.

In a recent study conducted by Dow Chemical (Stott et al., 1981) male B6C3F1 mice had a greater capacity to metabolize TCI than did male Osborne-Mendel rats. Both species were exposed by inhalation for 6 hours. Mice metabolized 2.2 times more TCI per kg body weight after exposure to 10 ppm and 3.6 times more TCI per kg body weight after exposure to 600 ppm than did rats. The data indicated saturation of TCI metabolism in rats at the higher level of exposure. Macromolecular binding studies showed that the mice activated more TCI to a reactive metabolite than bound to macromolecules in liver and kidney than did rats and that little of the binding involved alkylation of DNA. The authors point out that "the DNA alkylation data indicate a relative lack of genotoxic potential."

The problems encountered with balance studies in man are the difficulties associated with accurate measurement of the retained dose of TCI from vapor exposure, and the imprecision of the older methodologies for determinations

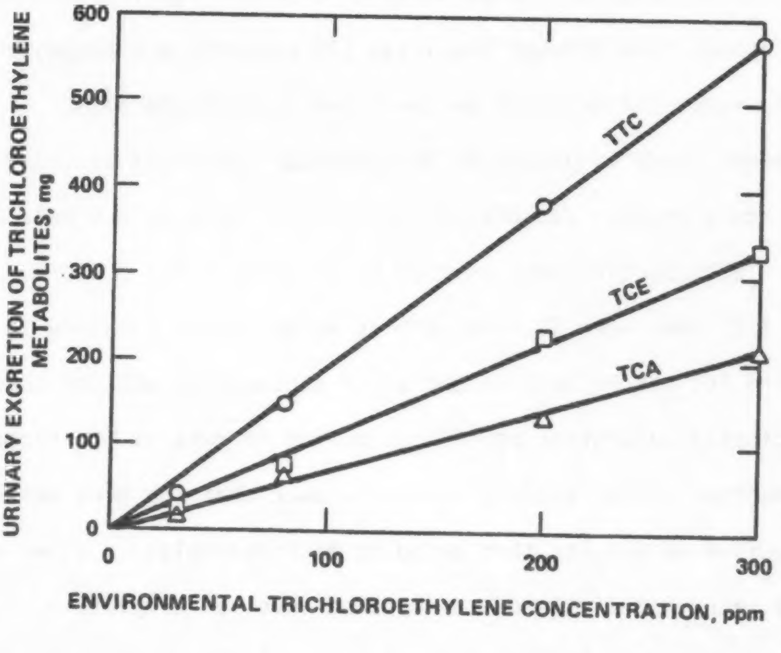


Figure 4-3. Relationship between environmental trichloroethylene concentration and urinary excretion of trichloroethylene metabolites in human urine. Urine was collected for 6 days after exposure. (Adapted from Nomiyama and Nomiyama, 1977).

of urinary metabolites by extraction and colorimetry using modifications of the Fujiwara reaction. More recent careful studies using GC methods show that after single or repeated daily exposures from 50 to 380 ppm, an average of 11 percent of retained TCI is eliminated unchanged by the lungs (t 1/2, 5 hr), 2 percent of the dose is eliminated as TCE by the lungs (t 1/2, 10 to 12 hr), and 58 percent is eliminated as urinary metabolites (Fernandez et al., 1975, 1977; Muller et al., 1974; Monster et al., 1976, 1979). Unaccounted for is nearly 30 percent of the TCI dose. These investigators suggest additional pathways or routes of elimination of one or more unknown metabolites. Other reported but unconfirmed minor urinary metabolites of TCI such as chloroform (1 percent in rats, Muller et al., 1974) and monochloroacetic acid (4 percent in man, Soucek and Vlachova, 1960) do not account for the discrepancy. On the other hand, early studies in dogs by Owens and Marshall (1955) show that TCE-glucuronide is concentrated and secreted in bile. Several studies also show that TCE or TCA given orally or intravenously is not fully recovered in the urine (Marshall and Owens, 1954; Owens and Marshall, 1955; Paykoc and Powell, 1945; Muller et al., 1974). Bartonicek (1962) found that 8.4 percent of a TCI dose is excreted in the feces as TCE and TCA. Part of the unaccounted TCI dose may also be explained by flatus excretion; TCI can be expected to be rapidly distributed into the GI lumen because of its high lipid solubility and ubiquitous distribution. In any case, these studies indicate at least 60 percent and possibly as much as 90 percent of retained TCI from vapor exposures of less than 500 ppm is metabolized in the body.

4.7 TCI METABOLISM AND DRUG INTERACTIONS

A number of commonly used drugs might be expected to modify the extent of metabolism of TCI during human exposure. The converse may also occur, resulting in important modifications of the therapeutic actions of drugs.

Although largely undocumented in man, the induction of hepatic microsomal mixed function oxidate system by drugs taken for therapeutic reasons or by exposure to certain environmental chemicals (e.g., barbiturates, PCBs, etc.), may bring about an increased rate of TCI metabolism. Numerous _in vivo_ and _in vitro_ experimental studies with animals pretreated with microsomal inducers have demonstrated an enhanced metabolism of TCI (Leibman and McAllister, 1967; Carlson, 1974; Moslen et al., 1977a,b; Pessayre et al., 1979). Conversely, TCI competitively inhibits the metabolism of barbiturates, producing exaggerated effects of these drugs (Kelley and Brown, 1974).

A drug interaction has been shown to occur in persons exposed to TCI who are also on a therapeutic regimen of disulfiram for alcoholism. Disulfiram inhibits the oxidation of acetaldehyde by competing with NAD for active sites on aldehyde dehydrogenase. Bartonicek and Teisinger (1962) showed that disulfiram markedly inhibits the terminal enzymatic steps of TCI metabolism in man. Since these steps, which involve the conversion of chloral hydrate to TCE and TCA (Figure 4-2), can be considered detoxification mechanisms, disulfiram has the potential of enhancing TCI and chloral hydrate toxicities. Sellers et al. (1972b) investigated the interaction of ethanol and chloral hydrate metabolisms. These investigators observed that concomitant administration of alcohol and chloral hydrate in man exacerbated the side effects of chloral hydrate (vasodilation, tachycardia, facial flushing, headache and hypotension). Their patients also exhibited impairment of auditory function and of performance of complex motor tasks. Similar effects have been observed during acute and chronic TCI exposure and concomitant ingestion of alcohol (Müller et al., 1975).

Stewart et al. (1974) have described the phenomenon of "degreasers" flush (facial and upper extremity skin vasodilatation) and determined that this effect of alcohol interaction with TCI persists after cessation of exposure. The intolerance syndrome is ascribed to mutual inhibition of TCI and alcohol metabolisms producing increased plasma ethanol and TCE concentrations from competitive inhibition of aldehyde dehydrogenase (Sellers et al., 1972a,b; Stewart et al., 1974).

Müller et al. (1975) specifically investigated the interaction of TCI and alcohol in human volunteers. After inhaling TCI (50 ppm) for 6 hours per day for 5 days, ethanol ingestion inhibited TCI metabolism to TCE and TCA by 40 percent. Blood TCI concentration increased more than 2-fold. This latter observation suggested that alcohol also inhibited, by competition, the microsomal oxidation of TCI to chloral hydrate. These investigators proposed that the intolerance syndrome from combined exposure to TCI and ethanol was due to increased accumulation of TCI in the CNS resulting from depression of TCI oxidation. From experiments in rats, Nakanishi et al. (1978) found that ethanol ingested after TCI exposure increased blood acetaldehyde level 3 to 4 fold. They suggest that TCI inhibits acetaldehyde dehydrogenase, and the resulting increase of blood levels of acetaldehyde are responsible for the intolerance syndrome.

Sellers and Koch-Weser (1970) observed a potentiation of the anticoagulant effect of Warfarin in patients after chloral hydrate ingestion. This increased potential for bleeding appeared to result from displacement of Warfarin from plasma protein binding sites by the chloral hydrate metabolite, TCA. Considering the significant amounts of TCA formed after TCI exposure and the long half-time of excretion of TCA, it is likely that TCI may potentiate the effects of many other drugs which normally bind to the same plasma protein sites as TCA.

4.8 METABOLISM AND TOXICITY

The metabolism of TCI has been suggested as a possible vector for the hepatotoxicity and nephrotoxicity associated with excess human exposure to this haloalkene (James, 1963; Defalque, 1961; Smith, 1966; Litt and Cohen, 1969; Baerg and Kimberg, 1970). Experimentally, TCI has been shown to be hepatotoxic in dogs and rats (Orth and Gillespie, 1945; Klaassen and Plaa, 1967; Cornish and Adefuin, 1966; Carlson, 1974; Cornish et al., 1977; Reynolds and Moslen, 1977). Reynolds and Moslen (1977), on physicochemical and biological correlative bases, have proposed that unsymmetric chlorinated ethylenes like TCI are more hepatotoxic than symmetric ethylenes as perchloroethylene and more so than vinyl chloride. The mechanism for TCI hepatotoxicity is not definitively established, but many investigators have proposed that the observed centrilobular cellular necrosis results from the microsomal mixed function oxidase system formation of chemically "reactive" metabolites which react with and bind to tissue macromolecules and cause intracellular damage (Pessayre et al., 1979; Allemand et al., 1978; Reynolds and Moslen, 1977). Figure 4-1 indicates that these reactive metabolites may include trichloroethylene epoxide, carbonium ions (alkylating agents) and possibly dichloroacetyl chloride. Chloral, chloral hydrate and trichloroethanol, as ordinarily used hypnotics, are not associated with significant hepatotoxicity (Goodman and Gilman, 1975).

Presently there is little information about the degree of binding of "reactive" metabolites to macromolecules that results in a toxic liver response, nor have the critical target binding sites been identified. However, numerous factors such as species, age, sex and diet, all of which modify TCI microsomal metabolism, also markedly influence the toxic response. Drugs such as phenobarbital (Carlson, 1974) and ethanol (Cornish and Adefuin, 1966) greatly influence TCI metabolism and hepatotoxicity. Cornish et al. (1977) found that TCI-induced liver toxicity in rats, as measured by plasma SGOT, is potentiated by both ethanol and phenobarbital. Ethanol acted by competitive substrate inhibition, and also by stimulation of microsomal mixed function oxidase system; phenobarbital potentiated toxicity by induction of mixed function oxidase system and thereby increased TCI metabolism. Allemand et al. (1978) observed a similar potentiation of toxicity in rats as measured by plasma SGPT. Plasma SGPT increase correlated with the amount of binding of ^{14}C-TCI reactive metabolites to liver microsomal protein. Reduced glutathione had a protective effect against TCI liver toxicity. TCI acutely administered to rats initially decreased hepatic glutathione by 61 percent, but increased glutathione levels (106 percent) 16 hours later (Moslen et al., 1977b). Glutathione added to microsomal preparations decreased binding of reactive products of ^{14}C-TCI, suggesting a glutathione conjugation of metabolites. Pessayre et al. (1979) found that single or acute TCI administration to rats decreased cytochrome P_{450} of pentobarbital-treated rats, but not untreated animals, and increased covalent binding of ^{14}C-TCI metabolites to microsomal protein. Chronic administration to normal rats simultaneously decreased P_{450} cytochrome content and increased ^{14}C-TCI metabolite binding, but also induced other microsomal enzyme systems. Since acute TCI exposure rarely produces functional liver impairment in man (Smith, 1966), whereas chronic exposure does (Litt and Cohen, 1969; Baerg and Kimberg, 1970), Pessayre and co-workers (1979) suggest that the explanation may lie in the ability of TCI to enhance its own metabolism and its own toxicity.

4.9 REFERENCES

Allemand, H., D. Pessayre, V. Descatoire, C. Degott, G. Feldman, and J-P. Benhamou. Metabolic activation of trichloroethylene into a chemically reactive metabolite toxic to the liver. J. Pharmacol. Exptl. Therap. 204:714-723, 1978.

Astrand, I., and P. Ovrum. Exposure to trichloroethylene. I. Uptake and distribution in man. Scand. J. Work Environ. & Health 4:199-211, 1976.

Baerg, R. D., and D. V. Kimberg. Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers." Ann. Intern. Med. 73:713-720, 1970.

Barrett, H. M., and J. H. Johnston. The fate of trichloroethylene in the organism. J. Biol. Chem. 127:765-770, 1939.

Barrett, H. M., J. C. Cunningham, and J. H. Johnston. Study of fate in organism of some chlorinated hydrocarbons. J. Indust. Hyg. Toxicol. 21:479-490, 1939.

Bartonicek, V. Metabolism and excretion of trichloroethylene after inhalation by human subjects. Brit. J. Ind. Med. 19:134-141, 1962.

Bartonicek, V., and J. Teisinger. Effect of tetraethyl thuiram disulphide (disulfiram) on metabolism of trichloroethylene in man. Brit. J. Ind. Med. 19:216-221, 1962.

Blair, A. H., and F. H. Bodley. Human liver aldehyde dehydrogenase. Partial purification and properties. Can. J. Biochem. 47:265-271, 1969.

Bolt, H. M., and J. G. Filser. Irreversible binding of chlorinated ethylenes to macromolecules. Environ. Health Persp. 21:107-112, 1977.

Bolt, H. M., A. Buchter, L. Wolowski, D. L. Gil, and W. Bolt. Incubation of ^{14}C-trichloroethylene vapor with rat liver microsomes. Uptake of radioactivity and covalent protein binding of metabolites. Int. Arch. Occup. Environ. Health 39:103-111, 1977.

Bonse, G., T. Urban, D. Reichart and D. Henschler. Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem. Pharmacol. 24:1829-1834, 1975.

Briemer, D., H. C. Ketclaars, and I. M. von Rossum. Gas chromatographic determination of chloral hydrate, trichloroethanol and trichloroacetic acid in blood and in urine employing head-space analysis. J. Chromatogr. 88:55-63, 1974.

Butler, T. C. Metabolic fate of chloral hydrate. J. Pharmacol. Exptl. Therap. 92:49-58, 1948.

Butler, T. C. Metabolic transformation of trichloroethylene. J. Pharmacol. Exptl. Therap. 97:84-92, 1949.

Buttner, H. Aldehyde and alcohol dehydrogenase activity in liver and kidney of the rat. Biochem. Z. 341:300-314, 1965.

Byington, K. H., and K. C. Leibman. Metabolism of trichloroethylene in liver microsomes. II. Identification of the reaction product as chloral hydrate. Mol. Pharmacol. 1:247-254, 1965.

Carlson, G. P. Enhancement of the hepatotoxicity of trichloroethylene by inducers of drug metabolism. Res. Commun. Chem. Path. Pharmacol. 7:637-640, 1974.

Cole, W. J., R. G. Mitchell, and R. F. Salamonsen. Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. J. Pharm. Pharmcol. 27:167, 1975.

Conkle, J.P., B.J. Camp, and B.E. Welch. Trace composition of human respiratory gas. Arch. Environ. Hlth. 30:290-295, 1975.

Cooper, J. R., and P. J. Friedman. The enzymic oxidation of chloral hydrate to trichloroacetic acid. Biochem. Pharmacol. 1:76-82, 1958.

Cornish, H. H., and J. Adefuin. Ethanol potentiation of halogenated aliphatic solvent toxicity. Am. Ind. Hyg. Ass. J. 27:57-61, 1966.

Cornish, H. H., M. L. Barth, and B. Ling. Influence of aliphatic alcohols on the hepatic response to halogenated olefins. Environ. Health Persp. 21:149-152, 1977.

Dalbey, W. and E. Bingham. Metabolism of trichloroethylene by the isolated perfused lung. Toxicol. Appl. Pharmacol. 43:267-277, 1978.

Daniel, J. W. The metabolism of ^{36}Cl-labelled trichloroethylene and tetrachloroethylene in the rat. Biochem. Pharmacol. 12:795-802, 1963.

Defalque, R. J. Pharmacology and toxicology of trichloroethylene; A critical review of the world literature. Clin. Pharmacol. Therap. 2:665-688, 1961.

Dowty, B.J., D.R. Carlisle, and J.L. Laseter. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environ. Sci. Technol. 9:762-765, 1975.

Eger, E. I., and C. P. Larson. Anesthetic solubility in blood and tissues; values and significance. Brit. J. Anaesth. 36:140-149, 1964.

Ertle, T., D. Henschler, G. Muller, and M. Spassowski. Metabolism of trichloroethylene in man. I. The Significance of trichloroethanol in long-term exposure conditions. Arch. Toxikol. 29:171-188, 1972.

Fernandez, J. G., B. E. Humbert, P. O. Droz, and J. R. Caperos. Exposition au trichlorethylene, Bilan de l'absorption, de l'excretion et du metabolisme sur des sujets humains. Arch. Mal. Prof. 36:(7-8)397-407, 1975.

Fernandez, J. G., P. O. Droz, B. E. Humbert, and J. R. Caperos. Trichloroethylene exposure. Simulation of uptake, excretion, and metabolism using a mathematical model. Brit. J. Ind. Med. 34:43-55, 1977.

Forssman, S., and C. E. Holmqvist. The relation between inhaled and exhaled trichloroethylene and trichloroacetic acid excreted in the urine of rats exposed to trichloroethylene. Acta Pharmacol et Toxicol. 9:235-244, 1953.

Friedman, P. J., and J. R. Cooper. The role of alcohol dehydrogenase in the metabolism of chloral hydrate. J. Pharmacol. Exptl. Therap. 129:373-376, 1960.

Fujiwara, K. Sitzungsker u. Abhandl naturforsch. Ges. Postock. 6:33, 1914.

Goodman, L. S., and A. Gilman. The Pharmacological Basis of Therapeutics. 5th ed. Macmillan, New York, 1975.

Greim, H., G. Bonse, Z. Radwan, D. Reichert, and D. Henschler. Mutagenicity and chromosomal aberrations as an analytical tool for in vitro detection of mammalian enzyme-mediated formation of reactive metabolites. Arch. Toxicol. 39:159-169, 1977.

Grunett, N. Oxidation of acetaldehyde by rat liver mitochondria in relation to ethanol oxidation and the transport of reducing equivalents across the mitochondrial membrane. Eur. J. Biochem. 35:236-243, 1973.

Hall, K. D., L. N. Garlington, W. K. Nowill, and C. R. Stephen. The analysis of small concentrations of trichloroethylene vapor by interferometry. Anesth. 14:38-45, 1953.

Helliwell, P. J., and A. M. Hutton. Trichloroethylene anesthesia. I. Distribution in the fetal and maternal circulation of pregnant sheep and goats. Anaes. 5:4-15, 1950.

Henschler, D. Metabolism and mutagenicity of halogenated olefins - a comparison of structure and activity. Environ. Health Persp. 21:61-64, 1977.

Ikeda, M. Metabolism of trichloroethylene and tetrachloroethylene in human subjects. Environ. Health Persp. 21:239-245, 1977.

Ikeda, M., H. Ohtsuji, T. Imamura, and Y. Komoike. Urinary excretion of total trichloro-compounds, trichloroethanol, and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. Brit. J. Ind. Med. 29:328-333, 1972.

James, W. R. L. Fatal addiction to trichloroethylene. Brit. J. Ind. Med. 20:47-49, 1963.

Kelley, J. M., and B. R. Brown, Jr. Biotransformation of trichloroethylene. Intern. Anesthesiol. Clin. 12:85-92, 1974.

Kimmerle, G., and A. Eben. Metabolism, excretion and toxicology of trichloroethylene after inhalation. I. Experimental exposure on rats. Arch. Toxikol. 30:115-126, 1973a.

Kimmerle, G., and A. Eben. Metabolic studies of trichloroethylene. II. Experimental human exposure. Arch. Toxicol. 30:127-138, 1973b.

Klaassen, C. D., and G. L. Plaa. Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol. Appl. Pharmacol. 10:119-131, 1967.

Kline, S. A., and B. L. Van Duuren. Reaction of epoxy-1,1,2-trichloroethane with nucleophiles. J. Heterocycl. Chem. 14:455-458, 1977.

Kraemer, R. J., and R. A. Deitrich. Isolation and characterization of human liver aldehyde dehydrogenase. J. Biol. Chem. 243:6402-6408, 1968.

Leibman, K. C., and W. J. McAllister, Jr. Metabolism of trichloroethylene in liver microsomes. III. Induction of the enzymic activity and its effect on excretion of metabolites. J. Pharmacol. Exptl. Therap. 157:574-580, 1967.

Leibman, K. C., and E. Ortiz. Metabolism of halogenated ethylenes. Environ. Health Persp. 21:91-97, 1977.

Liebler, C. S., and L. M. DeCarli. Hepatic microsomes, a new site for ethanol oxidation. J. Clin. Invest. 47:62 (Abst), 1969.

Litt, K. F., and M. I. Cohen. Danger---vapor harmful. Spot-remover sniffing. N. Engl. J. Med. 281:543-544, 1969.

Lowry, L. K., R. Vandervort, and P. L. Polakoff. Biological indicators of occupational exposure to trichloroethylene. J. Occup. Med. 16:98-101, 1974.

Marshall, E. K., and A. H. Owens. Absorption, excretion and metabolic fate of chloral hydrate. Bull. Johns Hopkins Hosp. 95:1-18, 1954.

McConnell, G., D.M. Ferguson and C.R. Pearson. Chlorinated hydrocarbons and the environment. Endeavor 34:13-18, 1975.

Migdal, A., Graczyk, E., Obodecka, Z., and K. Piesiak. Biochemical and neurological studies in acute oral poisoning with trichloroethylene. Wiad. Lek. 24:1669-1673, 1971.

Monster, A. C., and G. Boersma. Simultaneous determination of trichloroethylene and metabolites in blood and exhaled air by gas chromatography. Int. Arch. Occup. Environ. Health 35:155-163, 1975.

Monster, A. C., G. Boersma, and W. C. Duba. Pharmacokinetics of trichloroethylene in volunteers; Influence of workload and exposure concentration. Int. Arch. Occup. Environ. Health 38:87-102, 1976.

Monster, A. C., G. Boersma, and W. C. Duba. Kinetics of trichloroethylene in repeated exposure of volunteers. Int. Arch. Occup. Environ. Health 42:283-292, 1979.

Morgan, A., A. Black, and D. R. Belcher. The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann. Occup. Hyg. 13:219-233, 1970.

Moslen, M. T., E. S. Reynolds, and S. Szabo. Enhancement of the metabolism and hepatotoxicity of trichloroethylene and perchloroethylene. Biochem. Pharmacol. 26:369, 1977a.

Moslen, M. T., E. S. Reynolds, P. J. Boor, K. Bailey, and S. Szabo. Trichloroethylene-induced deactivation of P-450 and loss of liver glutathione *in vivo*. Res. Comm. Chem. Path. Pharmacol. 16:109-120, 1977b.

Muller, G., M. Spassovski, and D. Henschler. Trichloroethylene exposure, and trichloroethylene metabolites in urine and blood. Arch. Toxicol. 29:335-340, 1972.

Muller, G., M. Spassovski, and D. Henschler. Metabolism of trichloroethylene in man. II. Pharmacokinetics of metabolites. Arch. Toxic. 32:283-295, 1974.

Muller, G., M. Spassovski, and D. Henschler. Metabolism of trichloroethylene in man. III. Interaction of trichloroethylene and ethanol. Arch. Toxicol. 33:173-189, 1975.

Nakanishi, S., E. Shiohara, M. Tsukada, H. Yamazaki, and K. Okumura. Acetaldehyde level in the blood and liver aldehyde dehydrogenase activities in trichloroethylene-treated rats. Arch. Toxicol. 41:207-214, 1978.

NIOSH, National Occupational Hazard Survey, Vol. III, Survey Analyses and Supplemental Tables. USDHEW, Cincinnati, 1977, pp. 2864-2866.

Nomiyama, K. Estimation of trichloroethylene exposure by biological materials. Intl. Arch. Arbeitsmed. 27:281-292, 1971.

Nomiyama, K., and H. Nomiyama. Metabolism of trichloroethylene in humans. Sex difference in urinary excretion of trichloroacetic acid and trichloroethanol. Intl. Arch. Arbeitsmed. 28:37-48, 1971.

Nomiyama, K., and H. Nomiyama. Respiratory retention, uptake and excretion of organic solvents in man. Int. Arch. Arbeitsmed. 32:75-83, 1974a.

Nomiyama, K., and H. Nomiyama. Respiratory elimination of organic solvents in man. Int. Arch. Arbeitsmed. 32:85-91, 1974b.

Nomiyama, K., and H. Nomiyama. Trichloroethylene metabolism in man and animals, with special reference to host and agent factors modifying the trichloroethylene metabolism. Ind. Environ. Xenobiot. 2:173-176, 1977.

Oesch, F. Mammalian epoxide hydrases. Inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica, 3:305-340, 1973.

Oesch, F., N. Kaubisch, D. M. Jerina, and J. M. Daly. Hepatic epoxide hydrase. Structure-activity relationships for substrates and inhibitors. Biochem. 10:4858-4866, 1971.

Ogata, M., Y. Takatsuka, and K. Tomokuni. Excretion of organic chlorine compounds in the urine of persons exposed to vapours of trichloroethylene and tetrachloroethylene. Brit. J. Ind. Med. 28:386-391, 1971.

Orth, O. S., and N. A. Gillespie. A further study of trichloroethylene anesthesia. Brit. J. Anaesth. 19:161-173, 1945.

Owens, A. H., and E. K. Marshall. Further studies on the metabolic fate of chloral hydrate and trichloroethanol. Bull. Johns Hopkins Hosp. 97:320-326, 1955.

Paykoc, Z. V., and J. F. Powell. The excretion of sodium trichloroacetate. J. Pharmacol. Exptl. Therap. 85:289-293, 1945.

Pelkonen, O., and H. Vainio. Spectral interactions of a series of chlorinated hydrocarbons with cytochrome P-450 of liver microsomes from variously-treated rats. FEBS Lett. 51:11, 1975.

Pessayre, D., H. Allemand, J. C. Wandscheer, V. Descatoire, J-Y Artigou, and J-P. Benhamou. Inhibition, activation, destruction and induction of drug-metabolizing enzymes by trichloroethylene. Toxicol. Appl. Pharmacol. 49:355-363, 1979.

Pfaffenberger, C. D., A. J., Peoples and H. F. Enos. Distribution of volatile halogenated organic compounds between rat blood serum and adipose tissue. Intern. J. Environ. Anal. Chem. 8:55-65, 1980.

Pfaffli, P., and A-L. Blackman. Trichloroethylene concentration in blood and expired air as indicators of occupational exposure. A preliminary report. Work-Environ-Health 9:140-144, 1972.

Powell, J. F. Trichloroethylene: absorption, elimination and metabolism. Brit. J. Ind. Med. 2:142-145, 1945.

Powell, J. F. Solubility or distribution coefficient of trichloroethylene in water, whole blood, and plasma. Brit. J. Industr. Med. 4:233-236, 1947.

Reynolds, E. S., and M. T. Moslen. Damage to hepatic cellular membranes by chlorinated olefins with emphasis on synergism and antagonism. Environ. Health Persp. 21:137-147, 1977.

Sato, A., and T. Nakajima. A structure-activity relationship of chlorinated hydrocarbons. Arch. Environ. Health 34:69-75, 1979.

Sato, A. and T. Nakajima. Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. Brit. J. Ind. Med. 35:43-49, 1978.

Sato, A., T. Nakajima, Y. Fujiwara, and N. Murayama. A pharmacokinetic model to study the excretion of trichloroethylene and its metabolites after an inhalation exposure. Brit. J. Ind. Med. 34:55-63, 1977.

Sellers, E. M., and J. Koch-Weser. Potentiation of Warfarin-induced hypoprothrombinemia by chloral hydrate. N. Eng. J. Med. 283:827-831, 1970.

Sellers, E. M., G. Carr, J. G. Bernstein, S. Sellers, and J. Koch-Weser. Interaction of chloral hydrate and ethanol in man. II. Hemodynamics and performance. Clin. Pharmacol. Ther. 13:50-58, 1972a.

Sellers, E. M., M. Lang, J. Koch-Weser, E. LeBlanc and H. Kalant. Interaction of chloral hydrate and ethanol in man. I. Metabolism. Clin. Pharmacol. Ther. 13:37-49, 1972b.

Seto, T. A., and M. O. Schultze. Determination of trichloroethylene, trichloroacetic acid, and trichloroethanol in urine. Analyt. Chem. 28:1625-1629, 1956.

Smith, G. F. Trichloroethylene. A Review. Brit. J. Ind. Med. 23:249-262, 1966.

Soucek, B., and D. Vlachova. Excretion of trichloroethylene metabolites in human urine. Brit. J. Ind. Med. 17:60-64, 1960.

Stewart, R. D., and H. C. Dodd. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin. Am. Ind. Hyg. Assoc. J. 25:439-446, 1964.

Stewart, R. D., C. L. Hake, and J. E. Peterson. Use of breath analysis to monitor trichloroethylene exposures. Arch. Environ. Health 29:6-13, 1974.

Stewart, R. D., H. C. Dodd, H. H. Gay, and D. S. Erley. Experimental human exposure to trichloroethylene. Arch. Environ. Health 20:64-71, 1970.

Stewart, R. D., H. H. Gay, D. S. Erley, C. L. Hake, and J. E. Peterson. Observations on the concentrations of trichloroethylene in blood and expired air following exposure of humans. Am. Ind. Hyg. Assoc. J. 23:167-172, 1962.

Stewart, R., C. Hake, and J. Peterson. "Degreasers flush": Dermal response to trichloroethylene and ethanol. Arch. Environ. Health 29:1-5, 1974.

Stott, W. T., J. F. Quast, and P. G. Watanabe. The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats as Related to Oncogenicity. Prepared for Chemical Manufactures Association. Washington, DC. Ref. No. TCE 2.0-PHKS/MACM. 33 pp.

Tanaka, S., and M. Ikeda. Method for determination of trichloroethanol and trichloroacetic acid in urine. Brit. J. Ind. Med. 25:214-219, 1968.

Toftgård, R., and J-A Gustafsson. Biotransformation of organic solvents: A review. Scand. J. Work Environ. Health 6:1-18, 1980.

Tottmar, S. O. C., H. Petterson, and K. H. Kiessling. The subcellular distribution and properties of aldehyde dehydrogenase in rat liver. Biochem. J. 135:577-586, 1973.

Traylor, P. S., W. Nastainczyk, and V. Ullrich. Conversion of trichloroethylene to carbon monoxide by microsomal cytochrome P_{450}. In: Microsomes and drug oxidations. Proceedings of the 3rd International Symposium, Berlin, 1976. Suppl. Biochem. Pharmacol., Pergamon, Oxford, 1977. pp. 615-621.

Tsuruta, H. Percutaneous absorption of trichloroethylene in mice. Ind. Health 16:145, 1978.

Uehleke, H., and S. Poplawski-Tabarelli. Irreversible binding of ^{14}C-labelled trichloroethylene to mice liver constituents in vivo and in vitro. Arch. Toxicol. 37:289-294, 1977.

Uehleke, H., S. Poplawski, G. Bonse, and D. Henschler. Spectral evidence for 2,2,3-trichloro-oxirane formation during microsomal trichloroethylene oxidation. Naunyn-Schmiedebergs Arch. Pharmacol. 293:(Suppl.) 253 (abst), 1976a.

Uehleke, H., S. Poplawski, G. Bonse, and D. Henschler. Spectral evidence for oxirane formation during microsomal trichloroethylene oxidation. Arch. Toxicol. 37:95-105, 1976b.

U.S. Occupational Safety and Health Administration. Air contaminants. US Code Fed. Regl., Title 29, part 1910 " 1000, pp. 612-614, 1978.

Van Duuren, B. L. Chemical structure, reactivity and carcinogenicity of halohydrocarbons. Environ. Health Persp. 21:17-23, 1977.

Van Dyke, R. Dechlorination mechanisms of chlorinated olefins. Environ. Health Persp. 21:121-124, 1977.

Van Dyke, R., and M. B. Chenoweth. Metabolism of volatile anesthetics. Anesth. 26:348, 1965.

Vesterburg, O., and I. Astrand. Exposure to trichloroethylene monitored by analysis of metabolites in blood and urine. J. Occup. Med. 18:224-226, 1976.

Vesterburg, O., J. Gorczak, and M. Krasts. Exposure to trichloroethylene. II. Metabolites in blood and urine. Scand. J. Work Environ. & Health 4:212-219, 1976.

Vignoli, L., Jouglard, J., Vignoli, P., and Terrasson de Fougeres, P. Acute intoxication by trichloroethylene ingestion. Med. Leg. Assicur. 18:789-798, 1970.

Vyskocil, J., and B. Polak. Acute trichloroethylene poisoning. Vnitr. Lek. 9:860-863, 1963.

Wiecko, W. Oral poisoning with trichloroethylene. Wiad. Lek. 19:1117-1118, 1966.

Withey, J. R., and B. T. Collins. Chlorinated aliphatic hydrocarbons used in the foods industry: The comparative pharmacokinetics of methylene chloride, 1,2-dichloroethane, chloroform, and trichloroethylene after i.v. administration in the rat. J. Environ. Pathol. Toxicol. 3:313-332, 1980.

5. TOXICOLOGICAL EFFECTS IN MAN AND EXPERIMENTAL ANIMALS

5.1 INTRODUCTION

The objective of this chapter is to present a review of the relevant literature on the toxicologic effects of trichloroethylene (TCI, 1,1,2-trichloroethylene) in animals and humans after acute, chronic, and experimental exposure. In addition to central nervous system (CNS) and cardiovascular effects, particular attention is paid to respiratory effects, hepatic and renal toxicity, and interactions with common drugs. The teratogenic, mutagenic, and carcinogenic potential aspects of TCI toxicity are extensively reviewed in Chapters 6 and 7 as well as in the Carcinogen Assessment Group Report included in the Appendix of this document.

Literature reports on the toxicity of TCI are somewhat discordant. While it is generally agreed that in man TCI exerts toxic effects on the central nervous and cardiovascular systems, as well as on the skin, liver, and kidneys, evaluations of the type and severity of these adverse effects, particularly following industrial exposure, are often sketchy and contradictory, especially in regard to latent toxic manifestations. Furthermore, it is often difficult to separate the effects of TCI exposure from those resulting from concomitant exposure to other chemicals in the work or ambient environment. Also, evaluating TCI toxicity in experimental studies is complicated by impurities in the commercially available TCI used in many investigations.

5.2 EFFECTS ON THE CENTRAL NERVOUS SYSTEM

5.2.1 Human Studies: Acute Inhalation Exposure

The first extensive study of the effects of TCI on the nervous system was that of Stuber in 1931. Stuber reviewed a total of 283 cases of TCI poisoning, including 26 fatalities. All cases had occurred in European industrial operations. Stuber reported that the toxic action of TCI primarily involved the central nervous system (CNS), although some effects were also observed in the gastrointestinal and circulatory systems. Adverse effects on the kidney were rare, and no cases were reported of injury to the liver. Among the CNS symptoms most often described were headache, dizziness, vertigo, tremors, nausea, vomiting, sleepiness, fatigue, a feeling and appearance of lightheadedness or "drunkenness" increasing to unconsciousness and, in some cases, to death. In addition to the general narcotic effect, specific paralysis of the trigeminal nerve was also reported (Plessner, 1915; Persson, 1934). This observation led to the early use of TCI in the treatment of trigeminal neuralgia.

Numerous case histories have subsequently been reported on the effects of acute exposure to high doses of TCI. The earlier studies reported on the toxicity of TCI following anesthesia, in particular the occurrence of trigeminal palsies (Humphrey and McClelland, 1944). Boulton and Sweet (1960) suggested that trigeminal palsies that occurred in 24 patients resulted from inhalation of dichloroacetylene or phosgene during closed-circuit TCI anesthesia. These compounds are formed when exhaled TCI is passed through soda lime to remove carbon dioxide. The formation of these impurities and their subsequent toxicity have resulted in a decline in the use of TCI as an anesthetic.

Kleinfeld and Tabershaw (1954) reported on five fatal cases of TCI poisoning among industrial workers following acute overexposure. This was the first compilation of TCI-related fatalities. Four of the workmen who had been employed in a degreasing operation continued to work despite complaints of nausea, drowsiness, dizziness, and vomiting. All died while at work or within a few hours after leaving work. Death was attributed to ventricular fibrillation.

St. Hill (1966) reported the case of a man who died following acute exposure to TCI while boiling out tanks in a ship's hold after an accidental leakage. The man was exposed for about 20 hours, during which time he complained of headache, dizziness, double vision, and paralysis of the face and neck muscles. Two other men who had worked with the victim suffered similar but less severe symptoms. Similar symptoms were reported by Tomasini and Sartorelli (1971) in a patient following chronic and acute overexposure to TCI during operation of a dry cleaning unit. The symptoms included symmetrical bilateral VIIIth cranial nerve deafness as well as cerebral cortical dysrhythmia and alterations in the electroencephalogram (EEG). This patient recovered after exposure was stopped.

Loss of special senses has also been reported following TCI exposure. Mitchell and Parsons-Smith (1969) reported the case of a worker who lost his sense of taste after one month's exposure to high concentrations of TCI. He subsequently developed trigeminal analgesia and cortical changes reflected in alterations in the EEG.

Maloof (1949) reported blurring of vision and diplopia in a worker the day after he had been exposed to hot TCI vapors. In addition to symptoms characteristic of visual effects and effects on the CNS, the worker received first and second degree burns to the skin and first degree chemical burns to the eyes.

Many other such case histories of acute TCI exposure have been reported with similar symptomatologies. Visual disturbances, mental confusion, fatigue, nausea, and vomiting have all been observed. The dangers of acute exposure to TCI may be accentuated by visual disturbances and by loss of coordination. This may lead to poor manual dexterity and unsafe mechanical operations (Patty, 1958).

TCI has also been reported to have some abuse potential. Cases of deliberate and repetitive inhalation have been reported by James (1963) and by Ikeda and Imamura (1973). Abuse of TCI has been associated with hepato-renal toxicity (Clearfield, 1970). Respiratory and cardiac failure is believed to be the cause of death after acute inhalation exposure.

5.2.2 Human Studies: Chronic Exposure

Chronic low level exposure to TCI results primarily in neurological and neuropsychiatric symptoms consisting of tremors, giddiness, increased lacrimation, reddening of skin, decreased sensitivity of the hands, alcohol intolerance, neuroanesthetic syndrome with anxiety states, bradycardia, and insomnia (Bardodej and Vyskocil, 1956). Other manifestations are not as well defined. The first published report of chronic occupational overexposure to TCI in the United States summarized ten individual cases occurring prior to 1944 (McNally, 1937; Quadland, 1944). The symptoms in all the cases were characteristic of CNS disturbances. No quantitative estimation of exposure levels was provided.

Kunz and Isenschmid (1935) reported the case of a worker who used TCI to remove diamond powder from the rolls of a jewelry roll. It was estimated that 100 to 300 ml of the solvent evaporated directly in front of his face daily. Blindness was preceded by increasing changes in color perception and vision.

Loss of tactile sense, decreased mobility, and the inability to grasp objects between the thumb and fingers was noted in six women working with TCI to remove wax remaining on optical lenses (Albahary et al., 1959).

5.2.3 Experimental Human Studies

5.2.3.1 Controlled Studies--Psychomotor function and subjective responses to TCI have been studied in short-term, controlled human clinical studies to determine the effects of TCI on the CNS. Kylin et al. (1967) measured optokinetic nystagmus in 12 human subjects exposed to 1000 ppm TCI for 2 hours. Although a significant decrease in the fusion limit was noted, this test was found not to be a sensitive indicator of the effects of TCI. Stopps and McLaughlin (1967) exposed one subject to four different concentrations of TCI (100 to 500 ppm) for 3 hours. They found no significant effect on psychomotor performance at the lowest concentration, but they found a progressive decrease as the TCI concentration was increased. This study, however, was limited by the use of only one subject and by the limited exposure period used in the experiment, which is not comparable to an 8-hour work day. In a larger study involving six male students exposed to two 4-hour exposures in one day at an average concentration of 110 ppm, Salvini et al. (1971) demonstrated a statistically significant decrease in performance ability for tests of perception, manual dexterity, reaction time, and Wechsler memory scale.

The effects of TCI on visual motor performance as determined by psychophysiological tests were studied in 12 subjects by Vernon and Ferguson (1969). They found a significant decrease in performance only at the highest concentration tested (1000 ppm) after a 2-hour exposure.

Stewart et al. (1970) experimentally exposed ten subjects to 100 and 200 ppm TCI for periods ranging from 1 hour to a 5-day work week. They reported mild fatigue and sleepiness in normal adults after 4 to 5 days' exposure to 200 ppm TCI for 7 hours per day. Nomiyama and Nomiyama (1977) reported headaches in healthy male students exposed to about 100 ppm TCI for 4 hours per day for 6 days. No effects were observed on a flicker fusion or two-point discrimination test even at 200 ppm.

The short duration of many of these exposures decreases the value of these studies in assessing the longer-term, cumulative, and perhaps irreversible effects of TCI on nervous system function. They are useful primarily in measuring the threshold level for general CNS depressant activities of TCI.

5.2.3.2 Epidemiological Studies--Several epidemiological studies have been conducted in an attempt to correlate environmental concentrations and health effects. Grandjean (1963) found that 73 workers, exposed for 5 to 15 years to TCI concentrations of 1 to 333 ppm, exhibited alterations in autonomic nervous system function that were related to the length of time they were exposed to TCI; these changes were also accompanied by a high frequency of subjective symptoms. Bardodej and Vyskocil (1956) reported insomnia, tremors, and severe neuroasthenia syndromes coupled with anxiety states and bradycardia in 75 workers divided into four groups according to number of years they were exposed to TCI (5 to 629 ppm): less than 1 year, 1 to 2 years, 2 to 9 years, and over 10 years. They found that the symptoms increased in frequency with both longer exposures and higher concentrations of TCI. Disturbances of the nervous system were reported to continue for up to 1 year after final exposure.

In a detailed study of 104 workers exposed to TCI in metal, rubber, and dry cleaning industries, Andersson (1957) reported that two-thirds of the workers displayed CNS symptoms similar to those described for acute toxicity. Follow-up studies of the workers for 3 to 7 years after exposure showed little residual evidence of TCI intoxication. Although the average exposure level in this study was 200 to 400 ppm, it should be noted that the levels of exposure were subject to many unmeasurable fluctuations.

Lilis et al. (1969) reported a study of 70 young workers (83% less than 30 years old) exposed for up to 6 years to variable concentrations of TCI. The subjects showed symptoms of CNS and cardiac disturbances. Although the authors estimated the TCI concentrations to be about 10 ppm, a direct relationship between the incidence and severity of symptoms and the duration of exposure was not established.

Konietzko et al. (1978) reported changes in EEGs that correlated with subjective toxic effects reported by workers exposed to open basins of TCI. Studies such as these are somewhat limited and thus provide only suggestive information. Due to lack of background data and the potential interaction of other chemicals, it is difficult to attribute the observed effects directly to TCI exposure.

5.2.4 Animal Studies

Studies of the effects of TCI on the nervous system of experimental animals have been confined to behavioral and histopathological aspects. The animal data have generally confirmed the reports on the CNS depressant activity of TCI observed in man at roughly equivalent exposures.

An earlier study by Taylor (1936) found no significant serious adverse effects in rats exposed to 500, 1000, and 2000 ppm TCI for 6 hours/ day, 5 days/week, for 6 months. At 3000 ppm only two of an original 6 mice survived. Dogs exposed to 2000 ppm TCI and guinea pigs exposed over 1100 hours to 1200 ppm TCI showed no adverse effects.

In an early study, Baker (1958) investigated the histological changes in the CNS associated with acute and chronic exposure of dogs to TCI. The study was designed to produce graded exposures to TCI experimentally. Although no neuropathological changes could be detected after a single acute exposure to 30,000 ppm TCI, repeated exposure led to brain changes at scattered loci. Chronic exposure of dogs (2 to 8 hours/day, 5 days/week) to 300 to 500 ppm TCI resulted in selective destruction of the cerebellar Purkinje cells. Similar findings were reported by Bartonicek and Brun (1970) in rabbits chronically injected intramuscularly with a total of 53 g of TCI. The most pronounced changes appeared to occur in the Purkinje cells.

Recently, Tham et al. (1979) studied the effect of TCI on the vestibular system in rabbits by recording positional or rotational nystagmus. They found that at blood levels greater than 30 mg/liter (following continuous i.v. infusion of 1 to 5 mg/kg/min), TCI, unlike chloral hydrate and trichloroethanol, induced positional nystagmus. Their results suggested that TCI elicited vestibular disturbances by stimulating the central subcortical vestibulo-oculomotor connection.

Rats exposed to 9,000 ppm TCI for 15 minutes had slight difficulty in locomotion, but rats exposed to TCI levels of 12,000 to 15,000 ppm lost their righting reflex within a few minutes (Utesch et al., 1981). Cyclical acute exposure to 15,000 ppm TCI (5 minutes on, 15 minutes off), designed to simulate solvent abuse, produced no clinical chemistry or histopathologic evidence of renal or hepatic damage even after 5 hours of treatment. Concurrent oral administration of ethanol to the rats markedly potentiated the CNS depression produced by TCI but did not result in renal or hepatic damage (Utesch et al., 1981).

Behavioral techniques have also been used to study the effect of TCI and its metabolites in animals. Grandjean (1960) studied the effects of TCI on food-motivated conditioned responses in three rats. Exposure was via inhalation of 200 and 800 ppm TCI for 3 hours. He was unable to detect obvious behavioral changes related to TCI exposure, although there was some evidence of increased excitability or loss of inhibition. Battig and Grandjean (1963) chronically exposed rats to 400 ppm TCI for 10 months (8 hours/day, 5 days/week). Although exposure to TCI did not adversely affect their general condition, their swimming speed was reduced. They also found a significant increase in exploratory behavior, which confirmed their earlier studies (Grandjean, 1960). In a later study, Grandjean (1963) exposed rats for 6 hours to 400, 800, and 1600 ppm TCI. Although only slight decrements in swimming behavior were observed after 400 and 800 ppm, 1600 ppm produced significant decreases. This was also accompanied by evidence of fatigue at the higher dose. Goldberg et al. (1964) trained four rats in a conditioned avoidance escape test (pole climbing). Four-hour exposures, 5 days/week for 2 weeks to 200, 500, and 1500 ppm TCI did not result in altered responses. Learning appeared to be stimulated by 200 ppm exposure, while higher doses (4380 ppm) produced a specific inhibition of the avoidance response with no accompanying sign of motor imbalance. The responses did not increase or decrease directly with dose level. Silverman and Williams (1975), using observational methods, showed that repeated exposure (6 to 7 hours/day, 5 days/week, 4 weeks) of rats to 100 ppm TCI produced behavior changes in these animals. Although the changes were small and delayed, they were consistent with predictions derived from experiments with humans.

5.3 EFFECTS ON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

5.3.1 Cardiovascular Effects

The use of TCI as an anesthetic has been associated with cardiac arrhythmias including bradycardia, atrial and ventricular premature contractions, and ventricular extrasystoles. The dose-response relationships for these effects, however, has not been established in man or experimental animals. TCI has been reported to sensitize the myocardium to circulatory catecholamines resulting in cardiac arrhythmias including ventricular fibrillation or even cardiac arrest (Dhuner et al., 1951, 1957; Reinhardt et al., 1973). Metabolites of TCI, such as chloral hydrate, may also be associated with arrhythmias involving adrenergic stimulation (DiGiovanni, 1969). Lilis et al. (1969) observed in their study that 60% of the workers exposed to inhaled TCI exhibited an increase in cardiac output, an effect attributed to increases of epinephrine release and circulating plasma levels. These direct cardiac effects of TCI, with sensitization to catecholamines, have been observed after accidental ingestion and have, in some cases, proved fatal (Defalque, 1961).

Several case histories have been reported involving fatalities following acute exposure to large concentrations of TCI. The deaths in these cases were attributed to either cardiac arrest (James, 1963) or ventricular fibrillation (Kleinfield and Tabershaw, 1954). Bell (1951), Bardodej and Vyskocil (1956), Ogata et al. (1971), Andersson (1957), and others have noted that exposure to TCI may either speed up or slow down the heart rate depending on the degree of exposure. The most direct evidence that TCI causes ventricular extrasystoles, fibrillation, and cardiac arrest comes from changes that are observed in the electrocardiograms (EKG) of subjects accidentally exposed to TCI (Kledecki and Bura, 1963; Mroczek and Fedyk, 1971; Yacoub et al., 1973). Bernstine (1954) recorded ventricular fibrillation confirmed by EKG in a worker who sustained cardiac arrest after inhalation of TCI in analgesic concentrations. Barnes and Ives (1944) found that 33 of 40 normal patients in deep anesthetic planes showed signs of ventricular extrasystoles and multifocal ventricular tachycardia.

Few systematic animal studies have been done on the effects of TCI on the cardiovascular system. Mazza and Brancaccio (1967) studied the effects on rabbits of chronic exposures to 15,010 mg/m^3 (2790 ppm) TCI, 4 hours/day, 6 days/week, for 45 days. They found that severe blood dyscrasia was produced in these animals. The characteristics of the hemochromocytometric tests and the description of the bone marrow led the authors to conclude that chronic intoxication with TCI has a direct effect on the bone marrow which causes myelotoxic anemia. Mikiskova and Mikiska (1966) studied electrophysiological responses of guinea pigs injected intraperitoneally either with 6.7 mM/kg TCI or with 2.2 mM/kg trichloroethanol. They recorded EKG's and EEG's and found that trichloroethanol was at least three times as effective as TCI in inducing effects on the nervous system and in slowing the heart, and suggested that TCI effects were due, in part, to its metabolite, trichloroethanol.

5.3.2 Respiratory Effects

In anesthetic concentrations, TCI causes little or no irritation to the respiratory tract (Dobkin and Byles, 1963). A striking effect is observed, however, on the rate and depth of respiration. TCI characteristically causes increased respiratory rate (tachypnea) but decreased alveolar ventilatory amplitude, which is associated with decreased blood oxygen tension and increased carbon dioxide tension (Dobkin and Byles, 1963). These effects were noted in early animal experiments (Lehmann, 1911) and in early reports on human patients under TCI anesthesia (Striker et al., 1935). The incidence of tachypnea increases as the concentration of TCI increases (Atkinson, 1960).

To investigate the mechanism responsible for the increase in respiratory rate, Whitteridge and Bulbring (1944) studied the effects of TCI on pulmonary afferent nerve endings in cats. They found that 0.5-3.5 percent mg/m^3 (5,000-35,000 ppm) TCI produced a dose-dependent increase in the frequency of vagal discharge and an increased sensitivity of the stretch endings. Coleridge et al. (1968) observed similar results in isolated pulmonary receptor endings.

Stewart et al. (1970) attempted to correlate injury to rat pulmonary alveolar parenchyma with biochemical alterations following repeated inhalation of TCI. Mild changes in morphology were observed, including condensed mitochondria and vacuolation of the cells. Pulmonary surfactant secretion was significantly decreased. The authors suggested that modification of surfactant secretion was a consequence of nonspecific injury following exposure of a susceptible cell population to TCI.

5.4 HEPATIC AND RENAL TOXICITY

5.4.1 Human Studies

The similarity between TCI and chloroform and other related halocarbons known to cause liver damage suggests that TCI might possess hepatotoxic activity. Stuber (1931) reviewed 284 cases of industrial poisoning due to TCI and found no evidence of liver damage. Brittain (1948) found no evidence of liver or kidney damage in 250 neurosurgery patients who underwent prolonged TCI anesthesia. However, fatal acute hepatic failure has been observed following the use of TCI as an anesthetic. Such failure has generally been in patients with other complications such as malnutrition, toxemias and burns, or patients who had received transfusions (Ayre, 1945; Berleb, 1953; Dodds, 1945; Elam, 1949; Heizer, 1951; and Werch et al., 1955).

Secchi et al. (1968) reported acute liver disease in three of seven cases of poisoning following ingestion of TCI. They suggested, however, that the results could be attributed to contamination with 1,2-dichloropropane and 1,2-dichloroethane since no liver damage was found when pure TCI was ingested. Cotter (1960) described 10 patients acutely exposed to TCI in a confined space. Four patients showed hyperglobulinemia and six exhibited hypercalcemia. No other liver tests were abnormal and, in all but one person, the clinical tests returned to normal within two months of exposure. Lachnit and Brichta (1958) reported that of 22 workers occupationally exposed to TCI, only three had abnormal reactions on two liver function tests (sulfobromophthalein clearance tests and colloid stability). Albahary et al. (1959) conducted liver function tests--measuring serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)--on workers regularly exposed to TCI. No evidence of liver disorders was found. Guyotjeannin and Van Steenkiste (1958) found some abnormalities in cephalic flocculation, total lipids, and plasma unsaturated fatty acids, and an increase of plasma gamma globulins in 18 workers regularly exposed to TCI. Tolot et al. (1964) found no evidence of liver injury even though workers were routinely exposed to TCI at higher concentrations than the allowable tolerance limits: 0.9 to 3 mg of TCI per liter of air. Joron et al. (1955) reported massive liver necrosis followed by death more than one month after exposure to several hundred parts per million TCI.

From the above reports, it is apparent that observations of liver dysfunction in workers exposed to TCI have been infrequent, and relatively little surveillance has been conducted to ascertain latent effects. Thus, it is unlikely that the potential magnitude of any kidney or liver abnormalities in humans exposed for long periods to TCI is fully known. Cases of severe liver and kidney damage resulting from ingestion of liquid TCI may provide some evidence for latent toxicity. However, it is difficult to predict whether the same effects would be observed if ingestion of TCI was spread over a period of time similar to that found in inhalation exposure.

As mentioned earlier, TCI has also been reported to have some abuse potential. Cases of deliberate and repetitive inhalation have been reported by James (1963) and by Ikeda and Imamura (1973). Baerg and Kimberg (1970) documented centrilobular hepatic necrosis and acute hepatic and renal damage following repeated sniffing of cleaning fluid containing TCI and petroleum distillates by teenagers. However, as the vapor concentration was high and inhalation was only for a short period, the effect cannot be directly extrapolated to inhalation of the same total dose over a long period of time as in occupational exposure.

5.4.2 Animal Studies

Although chloroform and carbon tetrachloride display marked hepatotoxicity, results from animal experiments with TCI are still inconclusive. Early experiments showed some degree of liver damage. With two groups of dogs exposed to 750 ppm TCI 8 hours/day, 6 days/week, for 3 weeks, and 500 to 600 ppm TCI 6 hours/day, 5 days/week, for 8 weeks, Seifter (1944) found glycogen depletion and hydropic parenchymatous liver degeneration. Wirtschafter and Cronyn (1964) injected rats with TCI subcutaneously (0.5 g/kg); they found elevated SGOT levels within 12 to 16 hours which were correlated with hepatocellular damage observed in histological sections. This suggested a mild degenerative change of liver parenchyma. Gehring (1968) observed no significant increase in SGPT following a single exposure of mice to mg/m^3 (5500 ppm). Nowill et al. (1954) exposed one dog, three rabbits, and three rats to 0.05 to 0.1 percent mg/m^3 (500-1000 ppm) TCI for 18 hours/ day for 90 days. No gross abnormality was observed in liver, renal, or blood functions. Utesch et al. (1981) exposed rats intermittently to 15,000 ppm TCI in a manner simulating human solvent abuse. No evidence of liver or kidney damage was observed. Pendergast et al. (1967) reported negative observations following continuous exposure of rats, guinea pigs, dogs, rabbits, and monkeys to TCI. Ikeda et al. (1969) injected rats every 2 days for 37 days (0.5 ml/kg and 1.5 ml/kg). No significant changes were found in liver or blood lipids.

Liver failure in experimental animals is associated with binding of TCI metabolites to protein and nucleic acids in the liver (Bolt and Filser, 1977). Binding of TCI metabolites and TCI hepatotoxicity are increased by compounds that induce the liver mixed-function oxidase system and by treatment with inhibitors of epoxide hydrase. Adams et al. (1951) and Moslen et al. (1977) have found that TCI inhalation does not produce acute liver necrosis unless animals are pretreated with phenobarbital, especially when fasting. In addition, in phenobarbital-treated rats, hepatic glutathione contents decreased during TCI exposure, rebounding afterwards (Moslen et al., 1977). Despite the reports of hepatotoxicity in experimental animals exposed to high doses and in humans exposed to anesthetic doses of TCI, liver damage, similar to that observed in human industrial exposure, is rare (Bardodej and Vyskocil, 1956) and difficult to produce in experimental animals with acute subanesthetic doses (Kylin et al., 1963).

Dow Chemical (Stott et al., 1981) has recently performed gavage studies which include dosages similar to those used in the National Cancer Institute (NCI) (1976) bioassay for carcinogenicity of TCI by gavage in B6C3F1 mice and Osborne-Mendel rats (see Chapter 7). Male B6C3F1 mice were gavaged with 0, 250, 500, 1200, or 2400 mg TCI/kg body weight/day, 5 days a week, for 3 weeks. Male Osborne-Mendel rats, treated similarly, received 0 or 1100 mg TCI/kg body weight/day. Dose-related changes characteristic of hepatocellular hypertrophy were observed at all dosage levels in mice and ranged from slight histological changes at the lowest dose, to increased liver weights at 500 mg/kg, to increased centrilobular hepatocellular swelling at 1200 mg/kg, to severe centrilobular hepatocyte swelling, giant cell inflammation, and mineralized cells at the highest dose. No effect on the kidneys or on body weights was observed in either mice or rats. Rats treated with 1100 mg TCI/kg body weight had slightly increased liver wieghts but no histopathological changes in the livers. The authors suggest that the greater sensitivity of B6C3F1 mice to the hepatoxic effects of TCI reflects their greater capacity for metabolic activition of TCI (Section 4.6). From the results of their macromolecular binding experiments, including DNA alkylation studies (Section 4.6), and from these toxicity data, Stott and coworkers (1981) concluded that TCI induction of hepatic tumors in B6C3F1 mice may occur via an epigenetic mechanism.

Renal failure has been an uncommon problem with TCI anesthesia (Defalque, 1961). Although depressed kidney function has been observed in experimental animals exposed to TCI, it requires very high doses (Klassen and Plaa, 1967); on a relative basis, TCI is much less potent than chloroform or carbon tetrachloride.

5.5 DERMAL EFFECTS

Industrial use of TCI is often associated with dermatological problems (Bauer and Rabens, 1974). These include reddening and dermographic skin burns on contact (Maloof, 1949), generalized dermatitis resulting from contact with the vapor (McBirney, 1954), and possible scleroderma (Reinl, 1957). However, irritations, burns, and rashes are usually the result of direct skin contact with concentrated solvent and are, therefore, probably limited to effects secondary to the solvent. Stewart and Dodd (1964), in a study of controlled skin exposure (thumbs), showed that unless TCI is trapped against the skin, absorption is insignificant. Absorption of TCI varies with age, skin thickness and texture, as well as type of contact. No effects have been reported with exposure to TCI in dilute aqueous solutions.

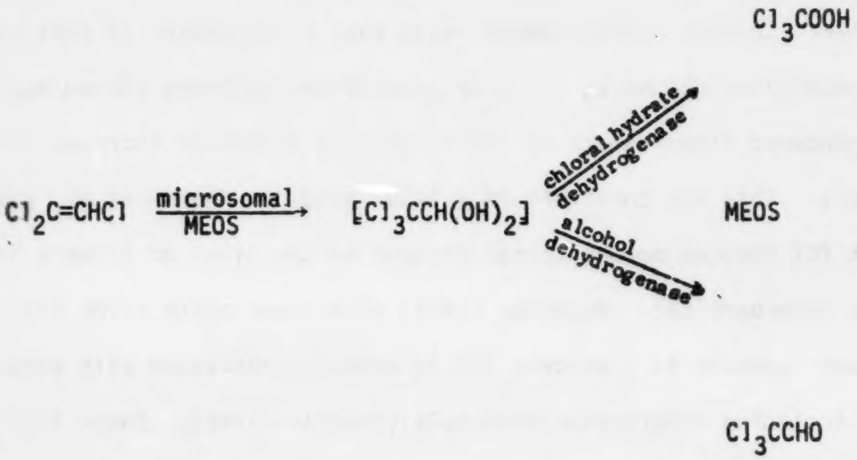
5.6 MODIFICATION OF TOXICITY: SYNERGISM AND ANTAGONISM

The long-term toxic effects of TCI appear to depend largely on its metabolic products. Therefore, drugs and chemicals that enhance or inhibit the metabolism of TCI will have a corresponding effect on the toxicity of TCI. Reynolds (1976) and Moslen et al. (1977) have reported that the hepatotoxic potential of TCI can be greatly enhanced by prior treatment with drugs or chemicals that induce components of the liver mixed-function oxidase (MFO) system. Carlson (1974) demonstrated that pretreatment of rats with phenobarbital (50 mg/kg i.p.) or 3-methylcholanthrene (40 mg/kg) exacerbated TCI-induced liver damage as indicated by a threefold increase in SGOT and SGPT levels. This was confirmed in a later study of Moslen et al. (1977) who found that TCI induced morphological changes in the liver of animals treated with only phenobarbital. Reynolds (1976) also found acute liver injury following a 2-hour exposure to 1 percent TCI in animals pretreated with other inducers of MFO including chlorinated biphenyls (Arochlor 1245). These findings suggest that a metabolite(s) of TCI contributes to the toxicity of the compound. In this regard, Mikiskova and Mikiska (1966) have found that trichloroethanol, a metabolite of TCI, was three to five times more effective in inducing changes in EKG and EEG.

Intolerance to alcohol is well characterized among TCI-exposed workers (Defalque, 1961). It is often manifested as red flushes on the face and upper parts of the body and has been referred to as "degreasers flush." Stewart et al. (1974) gave small oral doses of ethanol to seven subjects during exposure to 20, 100, and 200 ppm of TCI for 1, 3, or 7-1/2 hours. They reported a transient vasodilation of the superficial skin vessels which reached maximum intensity within 30 minutes. The metabolic sequences of TCI and ethanol are similar and they share some common enzymatic steps:

Ethanol Metabolism: $CH_3CH_2OH \xrightarrow[\text{dehydrogenase}]{\text{alcohol}} CH_3CHO \xrightarrow[\text{dehydrogenase}]{\text{aldehyde}} CH_3COOH$

ICI Metabolism:



$$\begin{tikzcd} & & Cl_3COOH \\ Cl_2C{=}CHCl \arrow[r, "\text{microsomal}", "\text{MEOS}"'] & {[Cl_3CCH(OH)_2]} \arrow[ur, "\text{chloral hydrate}", "\text{dehydrogenase}"'] \arrow[dr, "\text{alcohol}", "\text{dehydrogenase}"'] & \text{MEOS} \\ & & Cl_3CCHO \end{tikzcd}$$

Thus several interactions may occur. The effects depend on the dose and exposure levels. Cornish et al. (1977) found that simultaneous exposure to both ICI and ethanol resulted in substrate competition leading to altered excretion patterns of metabolites. Bardodej and Vyskocil (1956) suggested that ICI inhalation may result in inhibition of alcohol dehydrogenase, causing delayed ethanol metabolism and an increase in blood ethanol concentrations (Muller et al., 1975). Ethanol potentiation of the toxicity of ICI and its metabolites has been observed in both man (Bardodej and Vyskocil, 1956; Seage and Burns, 1971) and experimental animals (Cornish and Adefuin, 1966; Eerguson and Vernon, 1973; Gessner and Cabana, 1970; Utesch et al., 1981). Cornish and Adefuin (1966) reported that in rats the hepatotoxic response to short-term exposure (1.5 hours) to ICI (10,000 ppm) was markedly potentiated by prior ingestion of ethanol.

5.7 SUMMARY OF TOXIC EFFECTS

Toxicological effects on the central nervous system (CNS) and cardiovascular system, skin, liver, and kidney have been attributed to TCI exposure. Effects on the CNS, principally depression, have been well documented. Trigeminal palsies and visual deterioration have also been reported. Dermatological problems including irritation, burns, and rashes result from skin contact with the chemical. Fatal hepatic failure and renal failure have been observed following use of TCI as an anesthetic. In addition, cardiovascular and respiratory effects with a number of deaths attributed to respiratory arrest, cardiac arrhythmias including ventricular fibrillation, and primary cardiac standstill have resulted from acute exposures. Dose-response relationships for adverse health effects associated with TCI have not been established in man or experimental animals. Toxic dose data are presented in Table 5-1.

Intolerance to alcohol is a well-characterized phenomenon that results from TCI exposure. This effect has been reported to occur at TCI levels as low as 5 ppm. Classically, TCI is recognized as being a CNS depressant, and evidence exists for more persistent CNS effects resulting from higher level exposure, or more importantly, from lower-level chronic exposure. Psychomotor function and subjective responses which have been studied in short-term controlled human studies, as well as data from accidental exposure and epidemiological studies, indicate that nervous system function probably is affected by TCI concentrations ranging from 1 to 500 ppm. This evidence suggests that a threshold level for general CNS depressant activity or for acute effects of TCI is probably close to or below the established TLV and the OSHA standard of 535 mg/m^3 (100 ppm). Many other countries, especially several eastern European countries, have set much lower limits for occupational standards for TCI (Table 5-2).

TABLE 5-1. TOXIC DOSE DATA

Description of exposure[a]	Route of administration	Species	Dose (concn)	Toxic effect endpoint	Time
TCLo	Inhalation	Human	6,900 mg/m^3	Central nervous system effects[b]	10 min
TCLo	Inhalation	Human	160 ppm	Central nervous system effects[b]	83 min
TDLo	Inhalation	Human	812 mg/kg	Systemic effects[c]	--
TCLo	Inhalation	Man	110 ppm	Irritant effects[d]	8 hr
LCLo	Inhalation	Man	2,900 ppm	Death	--
LCLo	Inhalation	Rat	8,000 ppm	Death	4 hr
LCLo	Inhalation	Mouse	3,000 ppm	Death	2 hr
LCLo	Inhalation	Cat	32,500 mg/m^3	Death	2 hr
LCLo	Inhalation	Guinea pig (adult)	37,200 ppm	Death	40 min
LDLo	Oral	Human	7 g/kg	Death	--
LD50	Oral	Rat	4,920 mg/kg	Death	--
TDLo	Oral	Mouse	455 g/kg	Carcinogenesis	78 wk (intermittently)
LDLo	Oral	Dog	5,860 mg/kg	Death	--
LDLo	Oral	Cat	5,864 mg/kg	Death	--
LDLo	Oral	Rabbit (adult)	7,330 mg/kg	Death	--

TABLE 5-1. (continued)

Description of exposure[a]	Route of administration	Species	Dose (concn)	Toxic effect endpoint	Time
TD	Oral	Mouse	912 g/kg	Carcinogenesis	78 wk (intermittently)
LD50	i.p.	Mouse	3,000 mg/kg	Death	--
LD50	i.p.	Dog	1,900 mg/kg	Death	--
LD50	i.v.	Mouse	34 mg/kg	Death	--
LDLo	i.v.	Dog	150 mg/kg	Death	--
LDLo	Subcut.	Dog	150 mg/kg	Death	--
LDLo	Subcut.	Rabbit (adult)	1,800 mg/kg	Death	--
Skin and eye irritation data:					
--	--	Human	5 ppm	Eye irritation	--
		Rabbit (adult)	20 mg	Eye irritation	24 hr
--	--	Rabbit (adult)	500 mg	Skin irritation	24 hr

---

[a] In order to describe the dose, six abbreviations are used. These terms indicate whether the dose caused death (LD) or other toxic effects (TD) and whether it was administered as a lethal concentration (LC) or toxic concentration (TC) in the ihnaled air. In general, the use of the term "Lo" is used where the number of subjects studied was not a significant number from the population or the calculated percentage of subjects showing an effect was listed as 100. The definition of terms is as follows:

(continued)

TDLo - Toxic Dose Low - the lowest dose of a substance, as published or as made known to us, introduced by any route other than inhalation over any given period of time and reported to produce any toxic effect in humans or to produce carcinogenic, teratogenic, mutagenic, or neoplastigenic effects in humans or animals.

TCLo - Toxic Concentration Low - any concentration of a substance in air to which humans or animals have been exposed for any given period of time that has been reported to produce any toxic effect in humans or to produce a carcinogenic, teratogenic, mutagenic, or neoplastigenic toxic effect in animals or humans.

LDLo - Lethal Dose Low - the lowest dose of a substance other than LD50 introduced by any route other than inhalation over any given period of time and reported to have caused death in humans or animals introduced in one or more divided portions.

LD50 - Lethal Dose Fifty - A calculated dose of a chemical substance which is expected to cause the death of 50% of an entire defined experimental animal population, as determined from the exposure to the substance, by any route other than inhalation, of a significant number from that population. Other Lethal dose percentages, such as LD1, LD10, LD30, LD99, as well as others, may be published in the scientific literature for the specific purposes of the author. Such data would be published in the list if these figures, in the absence of a calculated lethal dose, such as LD50, LD1 and others, were the lowest published in the article.

LCLo - Lethal Concentration - the lowest concentration of a substance, other than an LC50, in air which has been reported to have caused death in humans or animals. The reported concentrations may be entered for periods of time which are less than 24 hours (acute) and greater than 24 hours (subacute and chronic).

LC50 - Lethal Concentration Fifty - a calculated concentration of a substance in air, exposure to which for a specified length of time caused the death of 50% of an entire defined experimental animal population as determined from the exposure to the substance of a significant number from that population.

[b] Central nervous system effects includes headaches, tremors, drowsiness, convulsions, hyponosis, anesthesia.

[c] Systemic effects - effects on metabolic and excretory function (liver or kidney).

[d] Irritant effects - any irritant effect on skin, eye, or mucus membrane.

Adapted from the NIOSH Registry of Toxic Effects of Chemical Substances, 1980.

TABLE 5-2. OCCUPATIONAL INDUSTRIAL HYGIENE STANDARDS FOR TRICHLOROETHYLENE

Country	ppm
USA	100
United Kingdom	100
Australia	100
Spain	100
Austria	50
Egypt	50
Federal Republic of Germany	50
Finland	50
Japan	50
Switzerland	50
Yugoslavia	50
German Democratic Republic	47
Czeckoslovakia CSSR	47
Sweden	30
Rumania	10
Hungary	10
Bulgaria	2
Poland	2
Soviet Union USSR	2

From: Fishbein, 1976; and Page and Arthur, 1978.

5.8 REFERENCES

Adams, E.M., H.C. Spencer, V.K. Rowe, D.D. McCollister, and D.D. Irish. Vapor toxicity of trichloroethylene determined by experiments on laboratory animals. Arch. Ind. Hyg. Occup. Med. 4:469-481, 1951.

Albahary, C., C. Guyotjeannin, A. Flaisler, and P. Thiaucourt. Transaminases et exposition professionnelle au trichloroethylene. Arch. Mal. Prof. Med. Trav. Secur. Soc. 20:421-426, 1959.

Andersson, A. Gesund heitliche Gefáhren in der Industrie bei Exposition fur Trichlorathylen. Health dangers in industry from exposure to trichloroethylene. Acta Med. Scand. Suppl. 157(323):1-220, 1957.

Atkinson, R.S. Trichloroethylene anaesthesia. Anesthesiology 21:67-77, 1960.

Aviado, D., S. Zakhari, J. Simaar, and A. Ulsamer. Methyl Chloroform and TCE in the Environment. CRC Press, Cleveland, OH, 1976.

Ayre, P. Acute yellow fever after Trilene anesthesia. Correspondence, Brit. M.J. 2:784, 1945.

Baerg, R.D., and D.V. Kimberg. Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers." Ann. Inter. Med. 73:713-720, 1970.

Baker, A.B. The nervous system in trichloroethylene: an experimental study. Neuropathol. Exp. Neurol. 17:649-655, 1958.

Bardodej, Z., and J. Vyskocil. The problem of trichloroethylene in occupational medicine: trichloroethylene metabolism and its effects on the nervous system as a means of hygienic control. AMA Arch. Ind. Health 13:581-592, 1956.

Barnes, C.G. and J. Ives. Electrocardiographic changes during trilene anesthesia. Proc. Roy. Soc. Med. 37:528-532, 1944.

Bartonicek, V.J., and A. Brun. Subacute and chronic trichloroethylene poisoning: a neuropathological study in rabbits. Acta Pharmacol. Toxicol. 28:359-369, 1970.

Battig, K., and E. Grandjean. Chronic effects of trichloroethylene on rat behavior. Arch. Environ. Hlth. 7:694-699, 1963.

Bauer, M., and S.F. Rabens. Cutaneous manifestations of trichloroethylene toxicity. Arch. Dermatol. 110:886-890, 1974.

Bell, A. Death from trichloroethylene in a dry-cleaning establishment. N.Z. Med. J. 50:119-126, 1951.

Bell, Z. Written communication with contractor reports of: (a) Dominant Lethal Study with Trichloroethylene in Albino Rats Exposed via Inhalation, February 9, 1977, and (b) Teratogenic Study via Inhalation with Trichlor 132, Trichloroethylene in Albino Rats, March 8, 1977.

Berleb, M., Eklampsie und Trichloran-Analgesie, München. Med. Wehnschr. 13: 1225, 1953.

Bernstine, M.L. Cardiac arrest occurring under trichloroethylene analgesia. AMA Arch. Surg. 68:262-266, 1954.

Bolt, H. M. and J. G. Filser. Irreversible binding of chlorinated ethylenes to macromolecules. Environ. Hlth. Persp. 21:107-112, 1977.

Boulton, T.B., and R.B. Sweet. The place of trichloroethylene in modern anesthesia. J. Mich. State Med. Soc. 59:270-273, 1960.

Brittain, G.J.C. Trichloroethylene (trilene) as anesthetic in neurosurgery. Anesth. & Analg. 27:145-151, 1948.

Carlson, G.P. Enhancement of the hepatotoxicity of trichloroethylene by inducers of drug metabolism. Res. Commun. Chem. Pathol. Pharmacol. 7:637-640, 1974.

Clearfield, H. Hepatorenal toxicity from sniffing spot remover (trichloroethylene). Dig. Dis. 15:851-856, 1970.

Coleridge, H.M., J.C.G. Coleridge, J.C. Luck and J. Norman. The effect of four volatile anaesthetic agents on the impulse activity of two types of pulmonary receptor. Br. J. Anaesth. 40:484-492, 1968.

Corbett, T., G. Hamilton, M. Yoon, and J. Endres. Occupational exposure of operating room personnel to trichloroethylene. Can. Anesth. So. J. 20:675-678, 1973.

Cornish, H.H., and J. Adefuin. Ethanol potentiation of halogenated aliphatic solvent toxicity. Am. Ind. Hyg. Assoc. J. 27:57-61, 1966.

Cornish, H.H., M.L. Barth, and B. Ling. Influence of aliphatic alcohols on the hepatic response to halogenated olefins. Environ. Hlth. Persp. 21:149-152, 1977.

Cotter, L.H. Trichloroethylene poisoning. Arch. Ind. Hyg. Occup. Med. 1:319-322, 1960.

Defalque, R.J. Pharmacology and toxicology of trichloroethylene: a review of the world literature. Clin. Pharmacol. Ther. 2:665-688, 1961.

Dhuner, K. G. Cardiac irregularities due to trichloroethylene given during labor. Acta Obst. Ct Gynee. Scandinav. 31:478-482, 1951.

Dhuner, K. G. Cardiac irregularities in trichloroethylene poisoning. Acta. anaesth. Scandinav. 1:121-135, 1957.

DiGiovanni, A. J. Reversal of chloral hydrate. Associated cardiac arrhythmia by a bita-adrenegic blocking agent. Anesthesiology 31: 93, 1969.

Dobkin A., and P. Byles. Trichloroethylene anesthesia. Clin. Anesth. 1:44-65, 1963.

Dodds, G. H. Necrosis of liver and bilateral massive suprarenal hemorrhage in puerperium, Brit. M. J. 1:769-770, 1945.

Elam, J. Correspondence. Brit. M. J. 1:546-547, 1949.

Ferguson, R.K., and R.J. Vernon. Trichloroethylene in combination with CNS drugs. Effects on visual-motor tests. Arch. Environ. Hlth. 2:462-467, 1973.

Fink, R. Toxicity of Anesthetics, Williams and Wilkins Co., Baltimore, MD. p. 270, 1968.

Fishbein, L. Industrial mutagens and potential mutagens. I. Halogenated aliphatic derivatives. Mutat. Res. 32:267-, 1976.

Gehring, P.J. Hepatotoxic potency of various chlorinated hydrocarbon vapors relative to their narcotic and lethal potencies in mice. Toxicol. Appl. Pharmacol. 13:287-298, 1968.

Gessner, P.D., and B.E. Cabana. A study of the interaction of the hypnotic effects and of the toxic effects of chloral hydrate and ethanol. J. Pharmacol. Exp. Therap. 174:247-259, 1970.

Goldberg, M.E., H.E. Johnson, U.C. Pozzani, and H.F. Smyth, Jr. Effect of repeated inhalation of vapors of industrial solvents on animal behavior. I. Evaluation of nine solvent vapors and pole-climb performance in rats. Am. Ind. Hyg. Assoc. J. 25:369-375, 1964.

Grandjean, E. Trichloroethylene effects on animal behavior. Arch. Environ. Hlth. 1:106-108, 1960.

Grandjean, E. The effects of short exposures to trichloroethylene on swimming performances and motor activity of rats. Am. Ind. Hyg. Assoc. J. 24:376-379, 1963.

Guyotjeannin, C., and J. Van Steenkiste. Action of trichloroethylene on proteins and lipoproteins. Study of 18 workers working in polluted atmosphere. Arch. Mal. Prof. Med. Trav. Secur. Soc. 19:489-494, 1958.

Heizer, H. Trichloraethylen in der Gebutshilfe, München. Med. Wehnschr. 93:2046-2050, 1951.

Humphrey, J.H., and M. McClelland. Cranial-nerve palsies with herpes following general anaesthesia: a report. Br. Med. J. 1:315-318, 1944.

Ikeda, M., and T. Imamura. Biological half-life of trichloroethylene and tetrachloroethylene in human subjects. Int. Arch. Arbeitsmed. 31:209-224, 1973.

Ikeda, T., C. Nagano, and A. Okada. Hepatotoxic effect of trichloroethylene and perchloroethylene in the rat and mouse. Igaku to Seibutsugaku 79:123-129, 1969.

James, W.R.L. Fatal addiction to trichloroethylene. Br. J. Ind. Med. 20:47-49, 1963.

Joron, G.E., D.G. Cameron, and G.W. Halpenny. Massive necrosis of the liver due to trichloroethylene. Can. Med. Assoc. J. 73:890-891, 1955.

Klassen, C.D., and G.L. Plaa. Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol. Appl. Pharmacol. 10:119-131, 1967.

Kledecki, Z., and H. Bura. Ostre zatrucia wywolane polknieciem trojehloroethylenu ("Tri"). Pol. Tyg. Lek. 18:748-750, 1963.

Kleinfeld, M. and I.R. Tabershaw. Trichloroethylene toxicity: report of five fatal cases. AMA Arch. Ind. Hyg. Occup. Med. 10:134-141, 1954.

Konietzko, H., W. Haberlandt, H. Heilbronner, G. Riell, and H. Weichardt. Chromosome studies on trichloroethylene workers. Arch. Toxicol. 40:201-206, 1978.

Kunz, E., and R. Isenschmid. The toxic effect of trichloroethylene on the eye. Klin Monatsbl Augenheilkd. 94:577-585, 1935.

Kylin, B., H. Reichard, I. Sumergi, and S. Yllner. Hepatotoxicity of inhaled trichloroethylene, tetrachloroethylene, and chloroform. Single exposure. Acta Pharmacol. Toxicol. 20:16-26, 1963.

Kylin, B., K. Axell, H.E. Samuel, and A. Lindborg. Effect of inhaled trichloroethylene on the CNS as measured by optokinetic nystagmus. Arch. Environ. Hlth. 15:48-52, 1967.

Lachnit, V., and G. Brichta. Trichloroethylene and liver damage. Zbl. Arbeitsmed. 8:56-62, 1958.

Lehmann, K.B. Experimental studies on the technical influence and essential hygienic gases and steam on the organism. Arch. Hyg. 74:1-60, 1911.

Lilis, R., D. Stanescu, N. Muica, and A. Roventa. Chronic effects of trichloroethylene exposure. Med. Lav. 60:595-601, 1969.

Maloof, C.C. Burns of the skin produced by trichloroethylene vapors at room temperature. J. Ind. Hyg. Toxicol. 31:295-296, 1949.

Mazza, V., and A. Brancaccio. Characteristics of the formed elements of the blood and bone marrow in experimental trichloroethylene intoxication. Folia. Med. 50:318-324, 1967.

McBirney, R.S. Trichloroethylene and dichloroethylene poisoning. AMA Arch. Ind. Hyg. Occup. Med. 10:130-133, 1954.

McNally, W.D. A case of phosgene poisoning due to the inhalation of decomposition products of trichloroethylene. Ind. Med. 6:539-544, 1937.

Mikiskova, H., and A. Mikiska. Trichloroethanol in trichloroethylene poisoning. Br. J. Ind. Med. 23:116-125, 1966.

Mitchell, A.B.S., and B.C. Parsons-Smith. Trichloroethylene neuropathy. Br. Med. J. 1:422-423, 1969.

Moslen, M.I., E.S. Reynolds, P.J. Boor, K. Bailey, and S. Szabo. Trichloroethylene-induced deactivation of cytochrome P-450 and loss of liver glutathione *in vivo*. Res. Commun. Chem. Pathol. Pharmacol. 16:109-120, 1977.

Mroczek, H., and I. Fedyk. Przpadek ciezkiego, samobojczego zatrucia trojechloroetylenem ("Tri"). Pol. Tyg. Led. 26:1509-1510, 1971.

Muller, G., M. Spassovski, and D. Henschler. Metabolism of trichloroethylene in man. III. Interaction of trichloroethylene and ethanol. Arch. Toxicol. 33:173-189, 1975.

NIOSH. Registry of Toxic Effects of Chemical Substances, 1980 update.

Nomiyama, K., and H. Nomiyama. Dose-response relationship for trichloroethylene in man. Int. Arch. Occup. Environ. Hlth. 39:237-248, 1977.

Nowill, W.K., C.R. Stephen, and G. Margolis. The chronic toxicity of trichloroethylene: a study. Anesthesiology 15:462-465, 1954.

Ogata, M., Y. Takatsuka, and K. Tomokuni. Excretion of organic chlorine compounds in the urine of persons exposed to vapours of trichloroethylene and tetrachloroethylene. Br. J. Ind. Med. 28:386-391, 1971.

Page, N. P. and J. L. Arthur. Special Occupational Hazard Review of Trichloroethylene. U. S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, January 1978.

Patty, E.A. Industrial Hygiene and Toxicology. 3 volumes, 2nd Revised Edition. Interscience Publishers, New York, NY, 1958.

Persson, H. On trichloroethylene intoxication. Acta Med. Scand. 59:410-422, 1934.

Plessner, W. On trigeminal disease due to trichloroethylene intoxication. Neurol. Zentr. 34:916-918, 1915.

Prendergast, J.A., R.A. Jones, L.J. Jenkins, Jr., and J. Siegel. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tretrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl. Pharmacol. 10:270-289, 1967.

Quadland, H.P. Petroleum solvents and trichloroethylene--Part 3 of the literature study of reports of occupational diseases attributed to volatile solvents. Ind. Med. 13:45-50, 1944.

Reinhardt, C. E.; L. S. Mullen; and M. E. Maxfield. Epinephrine-induced cardiac arrhythmia potential of some common industrial solvents. J. Occup. Med. 15:953, 1973.

Reinl, W. Scleroderma under the influence of trichloroethylene. Zentralbl. Arbeitsmed. 7:58-60, 1957.

Reynolds, E. A return to trichloroethylene. Br. J. Anaesth. 48:51 (Letter), 1976.

Salvini, M., S. Binaschi and M. Riva. Evaluation of the psychophysiological functions in humans exposed to trichloroethylene. Br. J. Ind. Med. 28:293-295, 1971.

Seage, A.J., and M.W. Burns. Pulmonary oedema following exposure to trichloroethylene. Med. J. Aust. 2:484-486, 1971.

Secchi, G.C., G. Chiappino, A. Lotto, and N. Zurlo. Actual chemical composition of commercial trilene and their hepatotoxic effects: clinical enzymologic study. Med. Lav. 59:486-497, 1968.

Seifter, J. Liver injury in dogs exposed to trichloroethylene. J. Ind. Hyg. Toxicol. 26:250-252, 1944.

Silverman, A.P. and H. Williams. Behaviour of rats exposed to trichloroethylene vapours. Br. J. Ind. Med. 32:308-315, 1975.

St. Hill, C.A. Occupation as a cause of sudden death. Trans. Soc. Occup. Med. 16:6-9, 1966.

Stewart, R.D., and H.C. Dodd. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin. Am. Ind. Hyg. Assoc. J. 25:439-446, 1964.

Stewart, R.D., H.C. Dodd, H.H. Gay, and D.S. Erley. Experimental human exposure to trichloroethylene. Arch. Environ. Hlth. 20:64-71, 1970.

Stewart, R.D., C.L. Hake, and J.E. Peterson. "Degreasers' flush": dermal response to trichloroethylene and ethanol. Arch. Environ. Hlth. 29:1-5, 1974.

Stopps, G.J., and M. McLaughlin. Psychophysiological testing of human subjects exposed to solvent vapors. Am. Ind. Hyg. Assoc. J. 28:43-50, 1967.

Stott, W. T., J. E. quast, and P. G. Watanabe. The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats as Related to Oncogenicity. Prepared for Chemical Manufactures Association. Washington, DC. Ref. No. TCE 2.0-PHKS/MACM. 33 pp.

Striker, C., S. Goldblatt, I.S. Wann, and D.E. Jackson. Clinical experience with use of trichloroethylene in production of over 300 analgesias and anesthesias. Anesth. and Analg. 14:68-71, 1935.

Stuber, K. Gesundheitsschadigen bei der Gewerblichen Verwendung des Trichloroalthylens und die Monglichkelien ihrer Verhutung. Arch. Gewerbepath. Bewerbehyg. 2:398-456, 1931.

Taylor, H. Experiments on the physiological properties of trichloroethylene. J. Ind. Hyg. Toxicol. 18:175-193, 1936.

Tham, R., B. Larsby, L. Odkvist, B. Norlander, D. Hyden, G. Aschan, and A. Bertler. The influence of trichloroethylene and related drugs on the vestibular system. Acta Pharmacol. Toxicol. 44:336-342, 1979.

Tolot, F., J. Viallier, A. Roullett, J. Rivoire, and J.C. Eigueres. Hepatic toxicity of trichloroethylene. Arch. Mal. Prof. Med. Trav. Secur. Soc. 25:9-15, 1964.

Tomasini, M., and E. Sartorelli. Chronic intoxication from commercial trielin inhalation with compromise of the eighth cranial nerves. Med. Lav. 62:277-280, 1971.

Utesch, R. C.; F. W. Weir; and J. V. Bruckner. Development of an animal model of solvent abuse for use in evaluation of extreme trichloroethylene inhalation. Toxicology 19(2):169-182, 1981.

Vernon, R.J., and R.K. Ferguson. Effects of trichloroethylene on visual-motor performance. Arch. Environ. Hlth. 18:894-900, 1969.

Werch, S. C., G. H. Marquardt, and J. F. Mallach. The action of trichloroethylene on the cardiovascular system. Am. J. Obst. and Gyn. 69:352-364, 1955.

Whitteridge, D., and E. Bülbring. Changes in activity of pulmonary receptors in anesthesia and their influence on respiratory behaviour. J. Pharmacol. Exptl. Therap. 81:340-359, 1944.

Wirtschafter, Z.I., and M.W. Cronyn. Relative hepatotoxicity. Arch Environ. Hlth. 9:180-185, 1964.

Yacoub, M., J. Faure, R. Rollux, J. Mallion, and C. Marka. L'intoxication aigue par le trichloroethylene. Manifestations cliniques et paracliniques, surveillance de l'elimination du produit. J. Eur. Toxicol. 6:275-283, 1973.

6. TERATOGENIC AND FETOTOXIC POTENTIAL OF TCI

As for any highly lipid soluble chemical that readily crosses the human placental barrier into the fetus (Helliwell and Hutton, 1950; Laham, 1970), trichloroethylene (TCI) warrants consideration as a possible fetal toxicant and potential teratogen. Also, TCI is known to be extensively metabolized in the maternal host by hepatic metabolizing enzymes (and possibly also in fetal liver) to chloral hydrate, and then to trichloroethanol and trichloroacetic acid (see Chapter 4, for details). These metabolites, particularly trichloroethanol, have also been shown to readily cross the human placenta into the fetal circulation and amniotic fluid (Bernstine and Meyer, 1953; Bernstine et al., 1954) and also into breast milk of nursing mothers (Berstine et al., 1956). Trichloroethanol has physical, chemical and pharmacologic properties similar to those of ethanol, which is known for its association with "fetal alcohol syndrome" (Clarren and Smith, 1978) and suspected to be a "direct acting " teratogen (Brown et al., 1979). There is little information available on the fetotoxicity and teratogenic potential of these metabolities. Chloral hydrate, and its metabolite trichloroethanol, have been in common medical use for a great many years as hypnotics (usual dose 0.5 to 2.0 gm), including use during pregnancy, but with no reported adverse teratogenic, fetotoxic, or reproductive effects (Goodman and Gilman, 1980). However, it should be noted that the chemical has not been evaluated for its ability to cause adverse effects in nonchemical exposure settings. Trichloroethanol has been administered to three animal species at various stages of pregnancy, at levels as high as 700 mg/kg/day, without dose-related effects (PDR, 1981).

This chapter reviews the teratology studies conducted with TCI, and assesses reports of fetal/neonatal toxicity in humans. These reports in humans provide circumstantial evidence only because the adverse teratogenic or reproductive effects of TCI that they describe are inadequate for scientific assessment since they consist of newspaper human interest stories, isolated clinical case reports, and clinical reports that do not have adequate exposure data. The review subscribes to the basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" as summarized and stated by Chernoff (1980):

> Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring *in utero*. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased incidence of supernumary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.
>
> In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to *in utero* exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.
>
> Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversiblity of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up". Unless a series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting after effects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/ fetal effect in one species might, under a given agent, react in another species in a more serious and irreversible manner.

6.1 ANIMAL STUDIES

Teratology studies that have been conducted on TCI are summarized in Table 6-1. The teratogenic and fetotoxicity potential of TCI has been evaluated in the avian embryo system, and in the rat, mouse and rabbit. These studies were conducted in accordance with current teratogenic testing methodology using, with one exception, technical grade TCI (Table 6-1).

6.1.1 Avian Embryo System

Fink in 1968, in describing the results from testing teratogenic potential of volatile anesthetic agents in the chick embryo, briefly mentioned that TCI in this system "showed a significant increase in fatalities and a slight increase in anomalies." Four groups of fertilized eggs totalling 96 embryos were exposed to 1% TCI vapor (10,000 ppm). Embyro blood concentration was serially determined during exposure but was no value was reported for TCI. The embryos were harvested on the third day of development, a time at which frank malformations would be difficult to observe or evaluate. Following this report, Elovaara et al. (1979) reported the results of a comparative study of the teratogenic potential of TCI and other aliphatic chlorinated hydrocarbons in the chick embryo. TCI in graded doses of 5 to 100 μmoles in olive oil (25 μl) were injected into the air space of fertilized eggs at 2 and 6 days of incubation and examined on the fourteenth day of incubation. Embryo toxicity was determined by incidences of survival and death after 14 days of incubation. The LD_{50} of TCI was between 50 and 100 μmoles/egg. Macroscopic embryonic malformations occured in 9 of 55 surviving fetuses, 5-fold in excess of the control vehicle. The teratogenic potential of TCI, in comparison to other halogenated hydrocarbon solvents, was evaluated as: 1,1,1 trichloroethane > trichloroethylene > methylene chloride, tetrachloroethylene, 1,1,2 trichloroethane > toluene > olive oil control.

TABLE 6-1. SUMMARY OF ANIMAL STUDIES OF FETOTOXIC AND TERATOGENIC POTENTIAL OF TCI

	TCI purity	Species	Conditions (mode of administration, dosage, and duration of exposure)	Measures	Results
Fink (1968)	Unknown	Chick embryo	Vapor exposure, 10,000 ppm	Mortality anomalies	Increase Slight increase
Elovaara (1979)	Reagent grade	Chick embryo	Injected; 5 - 100 μmole per egg in 25 μl olive oil	LD_{50},	50 - 100 μmole/egg (16% malformations in total survivors)
Schwetz et al. (1975)	Technical 99.24% TCI 0.76% stabilizers and impurities (Neu-tri®)	S - D Rat ♀ S - W mouse ♂	Inhalation; 300 ppm, 7h/d on 6 - 15d gestation	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)
Bell (1977)	Technical (Trichlor 132®)	CR - SD rat ♀	Inhalation; 300 ppm, 6h/d on 6 - 15d gestation	Embryo toxicity, teratogenicity, (maternal toxicity)	+ - (+)
Dorfmueller et al. (1979)	Technical 99%+ TCI 0.2% epichlorohydrin (Neu-tri®)	Rat ♀ (Long-Evans)	Inhalation, 1800 ppm a) Premating: 6h/d, 5d/wk for 2 wk b) Premating for 2 wk + first 20d gestation daily c) First 20d gestation, daily	Embryo toxicity, teratogenicity, offspring behavioral evaluation (maternal toxicity)	+ - (+)
Beliles et al. (1980)	Technical 99.9%	CR - SD Rat ♀ Rabbit ♀	Inhalation; 500 ppm, 7h/d 5d/wk Premating 3 wk + first 18d gestation daily (rat) + first 21d gestation daily (rabbit)	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)

At the present time, it is thought that disruption of normal embryonic differentiation in submammalian animal species (chick embryo) may be indicative of a potential for teratogenic effects in mammals; however, since there is a great deal of scientific uncertainty in this area, such studies are most appropriate for prescreening testing only (Johnson, 1981). Thus, avian studies provide information for comparing teratogenic effects between species, but no methodology exists for making extrapolations from submammalian species to humans (Karnofsky, 1965).

6.1.2 Mouse

Schwetz and his associates (Schwetz et al., 1975; Leong et al., 1975) investigated the potential of TCI to adversely effect fetal development in Swiss-Webster mice (Table 6-1). Twelve mice were exposed by inhalation to TCI at an air concentration of 300 ppm for 7 hours daily on days 6 through 15 of gestation. Day 0 of gestation was designated as the appearance of a vaginal plug. Concurrent pregnant control mice (30) were exposed to filtered air. The mice were observed daily throughout pregnancy, and maternal body weights were recorded on days 6, 10, 16 and 18 of gestation as an index of maternal toxicity. Following cesarean section on day 18, the fetuses were weighed, measured, (crown-rump length), sexed, and then examined for external anomalies. One-half of the fetuses were examined for soft tissue malformation using free-hand sectioning, and one-half of the fetuses were cleaned, stained, and examined for skeletal malformations. One fetus in each litter was subjected to microscopic examination.

Exposure to TCI had no effect on either the mean absolute or relative weight of the liver of mice dams when measured at the time of cesarean section. Exposure had no effect on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios or fetal body measurements. The incidence of gross anomalies observed by external examination was not significantly greater than among control litters. TCI exposure had no effect on the incidence of skeletal anomalies, and microscopic examination revealed no abnormalities of organs, tissues or cells. These investigators concluded that 300 ppm TCI caused no significant maternal, embryonal or fetal toxicity, and that TCI was not teratogenic in Swiss-Webster mice at this dose level.

6.1.3 Rat

Four studies conducted according to current teratology testing methodology, on two different strains of rats exposed by inhalation to 300, 500 and 1800 ppm TCI, have been conducted in this species (Table 6-1).

Schwetz and his associates (Schwetz et al., 1975; Leong et al., 1975) carried out a study in Sprague-Dawley rats. The design of the study was similar to their protocol for mice described above. TCI (300 ppm) was administered by inhalation, 7 hours daily, to 18 pregnant rats during organogenesis, the period when the greatest susceptibility to teratogenic effect is present (days 6 to 15 of gestation). Controls (30) were provided filtered air. Day 0 of gestation was defined as the day spermatozoa were observed in vaginal smears. The animals were killed on day 21 of gestation, cesarean sections were performed, and fetuses in each litter were examined for external malformations. One-half of the fetuses in each litter were then examined for soft tissue malformations, and the other half examined for skeletal malformations. One fetus from each litter was randomly selected for serial sectioning and histological evaluation. Schwetz et al. (1975) reported a small but statistically significant inhibition of maternal body weight gain when dams were exposed to 300 ppm TCI. However, this dose had no effect on either the mean absolute or relative weight of the maternal host livers when measured at the time of cesarean section. Exposure to TCI had no effect on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios or fetal body measurements. No increased incidence of soft tissue or skeletal anomalies were observed, and histopathological examination revealed no abnormalities of organs, tissues, or cells. Schwetz et al. concluded that 300 ppm TCI caused no significant maternal, embryonal or fetal toxicity in Sprague-Dawley rats.

Bell (1977) reported a similar study, conducted by Industrial Bio-Test Lab. Inc. (Northbrook, Ill.), on the teratogenic and fetal toxic effects of TCI upon gravid Charles River rats. TCI (300 ppm) was administered to 17 dams by inhalation exposure, 6 hours per day, from day 6 through day 15 of gestation. Control pregnant animals (21) were killed on day 20 of gestation. Cesarean sections were performed, fetal swellings and implantation sites counted, and resorption sites and number of corpora lutea determined. Viable fetuses were counted and all fetuses were removed and weighed. External examination of the fetuses were made for gross anomalies. Two-thirds of the fetuses from each litter were examined for skeletal development, and the remaining one-third of the fetuses were evaluated for soft tissue abnormalities. The investigators found that 300 ppm TCI exposure produced no evidence of maternal toxicity, although a small but significant reduction of mean weight gain by gestation day 15, in comparison to control dams, was recorded. There were no mortalities or adverse behavioral reactions, gross fetal anomalies, or differences in mean weights of fetuses exposed to TCI in comparison to unexposed controls. The numbers of corpora lutea, implantation sites, resorption sites, and fetuses were similar among exposed and control dams. No evidence for teratogenicity was observed in fetuses from either exposed or unexposed dams.

Beliles et al. (1980) reported on a study of teratogenic and fetotoxic potential of TCI in Sprague-Dawley rats conducted by Litton Bionetics, Inc. (Kensington, MD.). The experimental design of the study included pregestational as well as gestational exposure (Table 6-2).

TABLE 6-2. EXPERIMENTAL DESIGN OF BELILES et al. STUDY (1980) ON SPRAGUE-DAWLEY RATS

Group	No.	TCI ppm	Days of Exposure (Controls exposed to air only) Pre-gestational	Gestational
1	21	0 (control)	None	0-18
2	16	0 (control)	(5d/wk) 3 wk	0-18
3	20	500	None	0-18
4	16	500	(5d/wk) 3 wk	0-18
5	20	500	None	6-18
6	32	500	(5d/wk) 3 wk	6-18

Exposure to TCI, 500 ppm in air, was 7h/d, 5d/wk, during a 3 wk pre-gestational period, and 7 hr/d each day for days 0 to 18 and days 6 to 18 of gestation. Positive identification of spermatozoa in the vaginal canal was taken as evidence of mating and designated as day 0 of gestation. The dams were killed on day 20 of gestation, cesarean sectioned, the visceral and thoracic organs examined, and corpus lutea per dam determined. The maternal livers, kidneys and lungs were weighed. The number of implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were examined for gross external anomalies, weighed, and crown-rump length and sex determined. One-half of the fetuses of each litter were sectioned and examined for changes in the soft tissues; the remaining fetuses were examined for skeletal abnormalities.

The findings reported in this study indicate that maternal toxicity was not a factor in data interpretation; means of body weight, liver, kidney and lungs of test dams were within normal expected variation of control dams. No deaths occurred in the exposed groups. The investigators concluded that neither the frequency or character of the macro- or microscopic findings in the treated groups indicated an adverse effect on fetal growth and development, or a teratogenic potential for TCI at the dose level of 500 ppm.

Dorfmueller et al. (1979) conducted a study of the teratogenic and fetotoxic effects of TCI in Long-Evans rats exposed to a high inhalation concentration of 1800 ppm in air. Their experimental design was similar to that of Beliles et al. (1980) insofar as the animals were exposed during the pre-gestational period in addition to during gestation (Table 6-3).

The pre-mating exposure was conducted for 6 hr/d, 5d/wk for 2 wk, and the gestational exposure was 6 hr/d each day for days 0 - 20. Day 1 of pregnancy was considered to be the day on which sperm were observed in vaginal smears from mating. On day 21 of gestation, 15 out of 30 dams/treatment group were killed; maternal livers were removed, weighed and frozen for later enzyme analysis. Cesarean section was performed and the number of live, dead, and

TABLE 6-3. EXPERIMENTAL DESIGN OF DORFMUELLER et al. STUDY (1979) ON LONG-EVANS RATS

Group	No.	TCI ppm	Days of Exposure Pre-gestational	Days of Exposure Gestational
A	30	1800 ± 200	5d/wk; 2 wk	1 - 20
B	30	1800 ± 200	5d/ wk; 2 wk	none
C	30	1800 ± 200	none	1 - 20
D	30	none	none	none

resorbed fetuses counted. Each fetus was weighed, the sex recorded, and then examined for external anomalies. Four fetuses from each litter were examined for soft tissue changes, and four fetuses evaluated for skeletal abnormalities. Livers were removed from the remaining fetuses for measurement of mixed-function oxidase activity.

Postnatal behavioral evaluation was conducted on the offspring of the remaining 15 dams/treatment group which were allowed to litter. At weaning, 2 male, and 2 female pups from each litter were randomly selected for observation of activity measurements at 10 days of age (Motility Meter) and 20 and 100 days of age (Activity Cage).

Dorfmueller et al. (1979) found that the female rats exposed to 1800 ppm TCI before mating and/or during pregnancy did not exhibit any signs of maternal toxicity. Weight gains througout pregnancy were normal in relation to filtered air controls, and the relative and absolute maternal liver weights indicated no significant treatment effects occurred. There was no indication of embryotoxicity; no significant treatment effects or interactions were found in numbers of corpora lutea or implantation sites/ litter, fetal body weights, resorbed fetuses/litter, or sex ratios. No significant treatment effects were observed in the analysis of total soft tissue anomalies. However, the incidence of total skeletal anomalies was increased in group C, due to an increased incidence of incomplete ossification of the sternum, but when the frequency of this particular anomaly alone was compared in group C versus group D, the investigators found no significant difference. Incomplete ossification of the sternum is thought to be indicative of delays in the general process of skeletal ossification which are not necessarily irreversible. Thus, prenatal exposure to 1800 ppm TCI caused an elevation of minor anomalies indicative of development delays in maturation, but not of major malformations. Also, the study indicated no treatment effect on the general activity of the offspring at 10, 20 and 100 days of age. There was, however, a small but significant depression in postnatal weight gains of offspring in the premating exposure groups (A and B), the significance of which was not readily apparent. The offspring are being maintained until 18 months of age to further assess postnatal behavior and transplacental carcinogenicity potential. This study revealed no results indicative of treatment-related maternal toxicity, embryotoxicity, teratogenicity or significant postnatal behavioral deficits from exposure, pre-gestational and/or gestational, to 1800 ppm TCI in air.

6.1.4 Rabbit

Beliles et al (1980) also studied the fetotoxicity and teratogenicity of TCI in the female New Zealand White rabbit. Their experimental design is presented in Table 6-4.

Exposure to TCI, 500 ppm in air, was 7 hr/d, 5d/wk during a 3 wk pre-gestational period, and daily for days 0 to 21 and days 7 to 21 of gestation. Observed copulation and the presence of sperm in the vaginal canal established day 0 of gestation. On day 30 of gestation the dams were killed, cesarean

TABLE 6-4. EXPERIMENTAL DESIGN OF BELILES et al. STUDY (1980) ON NEW ZEALAND WHITE RABBITS

Group	Animals/ group	TCI ppm	Days of Exposure Pre-gestational	Days of Exposure Gestational
1	21	0 (control)	None	0-21
2	18	0 (control)	5d/wk; 3 wk	0-21
3	23	500	None	0-21
4	19	500	5d/wk; 3 wk	0-21
5	23	500	None	7-21
6	25	500	5d/wk; 3 wk	7-21

sectioned, and the corpora lutea per dam determined. The maternal livers, kidneys and lungs were removed, weighed and examined; the remaining visceral and thoracic organs were also examined. The number of uterine implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were examined for gross external anomalies, weighed, and then crown-rump length and sex ratios determined. One half of the fetuses of each litter were sectioned and examined for changes in the soft tissues; the remaining fetuses were examined for skeletal abnormalities.

The number of deaths in the TCI-treated groups was not significantly increased as compared to controls. Deaths occurring during the experiment account for the unequal numbers of animals in the groups. The mean body weights during pre-gestational and gestational periods were determined, and there were no remarkable differences between control and treated rabbits.

This study provided no evidence of maternal toxicity from 500 ppm exposure to TCI. Means of body weight, liver and lungs were within normal expected variation of control dams. A small increase in means of kidney weights for groups 2, 4, and 6 (compared to group 1) was found, but these increases were judged unrelated to treatment. No difference was observed in the mean weight of fetuses exposed•to TCI in comparison to those of unexposed controls. The number of corpora lutea, implantation sites, resorption sites, fetuses and sex ratio were similar among exposed and control dams.

Gross examination of fetuses with regard to external morphology and internal abnormalities revealed no unusual changes except for hydrocephalus in 4 fetuses of two litters of group 3. This anomaly occured only in the one treatment group; the malformation was not observed in the litters of rabbits that were exposed to 500 ppm TCI during a 3 week pre-gestational period plus the same gestational period (Group 4). The authors confirmed the anomaly as an external hydrocephalus, which in their experience, does not occur in untreated control rabbits. They, therefore, felt that, while not statistically significant, this finding could not be discounted entirely as occuring by chance. Unless other factors can be identified which are responsible for increasing the susceptibility of the rabbits during the gestational period, it would be expected that the malformation would occur in animals exposed to the same dose of TCI for a longer period.

The results of the examination of the fetuses for soft tissue anomalies showed no changes (except external hydrocephaly discussed above) that were not frequently observed in 30-day-old rabbit fetuses of the strain and source used in the study. Skeletal examination revealed no unusual features except for an increased incidence of changes related to retarded bone ossification, which were not considered malformations as such. Neither frequency nor the character of these changes in the treated groups indicated an adverse effect on fetal growth and development, or of a teratogenic potential. In summary, this study in rabbits exposed to 500 ppm TCI revealed no evidence of maternal toxicity or embryotoxicity; the authors did report the occurrence of hydrocephalus in a few fetuses of one of the study groups, but no definitive conclusion can be made regarding to this occurrence.

6.2 FETOTOXICITY IN HUMANS

There are no epidemiologic studies in the literature specifically directed to the investigation of fetotoxicity or overt birth anomalies that may occur from TCI exposure in the workplace. There are, however, scattered observations recorded in the literature and popular press that suggest an association between TCI exposure and fetotoxicity and/ or teratogenicity. Most of these reports are anecdotal in nature and at best provide only circumstantial evidence of a causal association between TCI exposure and fetal/neonatal toxicity in humans.

Corbett and his associates (1972; 1974) reported an increased incidence of miscarriages in operating room nurses exposed to various general anesthetics (volatile halogenated aliphatic hydrocarbons). TCI was only one of the anesthetics in concurrent use, and therefore it is impossible to make a specific association of TCI with the miscarriages. Furthermore, it was not possible to evaluate additive or synergistic effects of the various anesthetics, or other environmental stress factors. In addition, the study and its methodology has been severely criticized by Cote (1975), particularly with respect to clinical definition of anomalies and to methods of clinical evaluation.

Kucera (1968) reported a series of five cases of infants with sacral agenesis born to mothers exposed to various organic solvents during pregnancy. One of the mothers was exposed constantly to TCI and acetone. Further reports have not appeared in the literature since 1968 relating an association of this malformation and TCI, nor any other organic solvent, and therefore it is not possible to conclude that a causal relationship existed.

Jonak (1980) noted in a series of newspaper articles an incident involving an industrial spill of TCI for which air exposure levels were not measured but were estimated to have reached 12 times OSHA standards for TCI. The concentration of TCI was sufficient to have produced nausea and vomiting. Those exposed to TCI included two women each in the fourth month of their respective pregnancies. One of these women, 32 years old, and gravid with her second pregnancy, delivered a stillborn baby at normal term, without any external malformation. The second woman, gravid with her fifth pregnancy, gave birth at normal term to a child with "unexplained motor ability problems and slow mental and physical development." The child was reported to have improved with therapy, and at 8 months of age could hold his head up, sit up with assistance, reach for objects, but could not crawl. A medical history was not provided for either of these cases, and while the suggestion of fetotoxicity due to an acute exposure to a high concentration of TCI is possible, other causative or contributing factors cannot be ruled out.

Aviado et al. (1976) in his review concluded that TCI exposure may produce respiratory difficulties in neonates. However, respiratory effects on neonates has not been established and TCI has been in use for many years in Europe as a self-administered inhalant analgesic and anesthetic during labor without reported effects.

6.3 SUMMARY

The animal tests performed to date provide meager evidence of adverse reproductive or teratogenic effects of TCI. This chlorinated hydrocarbon is teratogenic in the chick embryo system, but there is no scientific consensus on the significance to humans of a positive finding in this test system. Well-designed inhalation exposure studies in the mouse (300 ppm), and rat (300,500, and 1800 ppm) by different investigators demonstrate no significant maternal toxicity, embryo toxicity, teratogenicity, or postnatal behavioral effects. In rabbits, 500 ppm inhalation exposure provide no evidence of maternal toxicity or embryo toxicity, but an assessment of teratogenic potential was made based on only a few hyrocephalic fetuses observed in one group (of four) exposed to TCI.

Anecdotal reports on the effects of acute, high-level exposure to TCI in humans suggest a possible association between TCI exposure and embryo/neonatal toxicity. However, these reports are not conclusive in establishing this association. The metabolites of TCI, chloral hydrate and trichloroethanol, are prescription drugs which have not been clinically associated with embryo/neonatal toxicity in the human.

At present, the available studies do not indicate that TCI is toxic to the fetus at levels which are below the maternally toxic level. Also, no definitive clinical evidence of fetotoxicity or teratogenicity from TCI exposure have been reported. Therefore, the available information does not indicate that the human conceptus is uniquely susceptible to the effects of TCI.

6.4 REFERENCES FOR CHAPTER 6

Aviado, D., S. Zakhari, J. Simaar, and A. Ulsamer. Methyl Chloroform and TCE in the Environment. CRC Press, Cleveland, OH, 1976.

Beliles, R. P., D. J. Brusick, and F. J. Mecler. Teratogenic-Mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene and carbon disulfide. U.S. Department of Health, Education and Welfare (Contract No. 210-77-0047), 1980.

Bell, Z. Written Communication with contractor reports of: (a) Dominant Lethal Study with Trichloroethylene in Albino Rats Exposed via Inhalation, February 9, 1977, and (b) Teratogenic Study via Inhalation with Trichlor 132,. Trichloroethylene in Albino Rats, March 8, 1977.

Bernstine, J. B. and A. E. Meyer. Passage of metabolites of chloral hydrate into amniotic fluid. Proc. Soc. Exp. Biol. Med. 84:456-457, 1953.

Bernstine, J. B., A. E. Meyer and R. L. Bernstine. Maternal blood and breast milk estimation following the administration of chloral hydrate during the puerperium. J. Obst. Gyn. 63:228-231, 1956.

Bernstine, J. B., A. E. Meyer and H. B. Hayman. Maternal and foetal blood estimation following the administration of chloral hydrate during labour. J. Obst. Gyn. 61:683-685, 1954.

Brown, N. A., E. H. Goulding and S. Fabro. Ethanol embryo toxicity: direct effects on mammalian embryos in vitro. Science 206:573-575, 1979.

Buelke-Sam, J., and C. A. Kimmel. Development and standardization of screening methods for behavioral teratology. Teratology 20:17-30, 1979.

Chernoff, N. Personal communication, and published in, U.S. EPA Office of Pesticides. Notice of determination: Abstract of Carbaryl Decesion Document. Federal Register 45:81869, December 12, 1980.

Christian, M. S. Dutchland Laboratories, Inc. Personnal communication, 1980.

Christian, M. S. Effects of maternal alterations on fetal development. The Toxicology Forum 1981 Annual Winter Meeting, Arlington, VA, pp:328-339, 1981a.

Christian, M. S. Postnatal alteration of gastrointestinal physiology, hematology, clinical chemistry and other non-CNS parameters. In: Handbook of Experimental Pharmacology. Johnson, E. M. and Kochhar, D. M., eds., Springer-Verlag, NY, 1981b. (in press).

Clarren, S. K. and D. W. Smith. The fetal alcohol syndrome. New Eng. J. Med. 298:1063-1067, 1978.

Corbett, T. H. Anesthetics as a cause of abortion. Fert. Steril. 23:866-869, 1972.

Corbett, T. H. R. G. Cornell, J. L. Endres and K. Leiding. Birth defects among children of nurse anesthetists. Anesthesiology 41:341-344, 1974.

Corbett, T., G. Hamilton, M. Yoon, and J. Endres. Occupational exposure of operating room personnel to trichloroethylene. Can. Anesth. Soc. J. 20:657-678, 1973.

Cote, C. J. Birth defects among infants of nurse anesthetists. Anesthesiology 42:514-515, 1975.

Dorfmueller, M. A., S. P. Henne, R. G. York, R. L. Bornschein, and J. M. Manson. Evaluation of Teratogenicity and Behavioral Toxicity with Inhalation Exposure of Maternal Rats to Trichloroethylene. Toxicol. 14:153-166, 1979.

Elovaara, E., K. Hemminki, and H. Vainio. Effects of methylene chloride, trichloroethane, trichloroethylene, tetrachloroethylene and toluene on the development of chick embryos. Toxicology 12:111-119, 1979.

Fink, R. Toxicity of Anesthetics. Williams and Wilkins Co., Baltimore, MD, 1968. pp. 270.

Goodman and Gilman's. The Pharmacological Basis of Therapeutics, editors, A. G. Gilman, L. S. Goodman and A. Gilman, 6th ed. New York: McMillan Pub. Co., 1980, pp. 361-363.

Helliwell, P. and M. Hutton. Trichloroethylene Anesthesia. I. Distribution in the fetal and maternal circulation of pregnant sheep and goats. Anaes. 5:4-15, 1950.

Johnson, E. M. Screening for teratogenic hazards, nature of the problems. Ann. Rev. Pharmacol. Toxicol. 21:417-429, 1981.

Jonak, D. Daily and Sunday Herald, (series of articles), Paddock Publications, Inc. Arlington Heights, IL, 1980.

Karnofsky, D. A. Drugs as teratogens in animals and man. Ann. Rev. Pharmacol. 5:447-472, 1965a.

Karnofsky, D. A. The chick embryo in drug screening; survey of teratological effects observed in the 4-day chick embryo. In: Teratology, Principles and Techniques, Wilson, J. G. and Warkany, J., eds. Un. Chicago Press, Chicago, IL, pp. 194-213, 1965b.

Kucera, J. Exposure to fat solvents: a possible cause of sacral agenesis in man. J. Pediatr. 72:857-859, 1968.

Laham, S. Studies on placental transfer: trichloroethylene. Ind. Med. 39:46-49, 1970.

Leong, B. K. J., B. A. Schwetz, and P. J. Gehring. Embryo- and fetotoxicity of inhaled trichloroethylene, perchloroethylene, methylchloroform and methylene chloride in mice and rats. Toxicol. Appl. Pharmacol. 33(1):136 (Abstract 34), 1975.

NTP Technical Bulletin, (December, 1980) 1(3):8.

PDR, Physician's Desk Reference, Oradell, N.Y.: Medical Economics Co., 1981, p. 1258.

Schwetz, B. A., B. K. J. Leong, and P. J. Gehring. The effect of maternally inhaled trichloroethylene, perchloroethylene, methylchloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol. Appl. Pharmacol. 32:84-96, 1975.

Skalko, R. G., and D. S. Packard, Jr. The incorporation of teratogenic agents into the mammalian embryo. In: Congenital Defects, Janerich, et al. Eds., Academic Press, NY, 1974. pp. 235-254.

Taylor, H. Experiments on the physiological properties of trichloroethylene. J. Ind. Hyg. Toxicol. 18:175-193, 1936.

York et al. The Toxicologist 1(1):28 (abstract), 1981.

7. CARCINOGENIC POTENTIAL

The discovery of the carcinogenicity of vinyl chloride, first in animals (Viola et al. 1971; Maltoni and Lefemine, 1975), and subsequently in humans (Creech and Johnson, 1974; Waxweiler et al. 1976), as well as the demonstration of mutagenic activity in bacterial systems (Greim et al., 1975), has had a definite effect on the value of laboratory bioassays, and has stimulated interest in research on structurally related compounds. Thus, the analogs of vinyl chloride, including trichloroethylene (TCI), have been intensively investigated for their mutagenic and carcinogenic potential. Like vinyl chloride (Laib et al., 1979; Fishbein, 1979), TCI has also been shown to be metabolized in mammalian organisms to epoxides and other highly reactive intermediates that covalently bind to cellular microsomal protein and lipids and to DNA (Van Duuren and Banerjee, 1976; Banerjee and Van Duuren, 1978; Bolt et al., 1977; Bonse et al., 1975; Uehleke and Poplawski-Iabarelli, 1977; see Chapter 4 for discussion). The chemical interactions of these metabolites with cellular macromolecules provides a theoretical basis for supposing TCI may be mutagenic and also have potential as a human carcinogen (Henschler and Bonse, 1977; Greim et al., 1975; Henschler et al., 1977).

The objective of this chapter is to summarize the more detailed review and evaluation of the literature presented in Appendix A relevant to the question of TCI carcinogenicity under the headings: (1) mutagenicity and cell transformation, (2) animal bioassays, and (3) epidemiologic studies. (See Appendix A for the complete report on mutagenicity and carcinogenicity prepared by the Carcinogen Assessment Group and the Reproductive Effects Assessment Group.) Waters et al. (1977) have reviewed animal studies in the older literature relating to the carcinogenic potential of TCI. Although their review was mainly concerned with the general toxic effects of TCI, they were unable to document a carcinogenic effect in any of a wide variety of animal species exposed to TCI. However, these toxicological studies involved small groups of animals and/or short durations of exposure. The International Agency for Research on Cancer has recently re-evaluated the potential cancer-causing activity of TCI and concluded that there is only limited evidence in mice that TCI is carcinogenic (IARC, 1979). In a more up-to-date comprehensive appraisal, the EPA Carcinogen Assessment Group (CAG, 1981) concluded that technical grade TCI and pure TCI have carcinogenic activity from evidence based on increased incidence of hepatocellular carcinomas in exposed B6C3F1 mice, positive mutagenic responses of bacteria and yeast and the mouse spot test, positive response to chloral hydrate in bacteria, a positive cell transformation study in Fischer F1706 rat embryo cells and/or increased the incidence of lymphomas in exposed female Han:NMRI mice. (See Appendix A for the complete Carcinogen Assessment Group report.)

The use of TCI having different grades of purity has been a complicating factor, impeding a definite interpretation of many of the mutagenic and carcinogenic bioassays of the compound. Technical, reagent, and analytical grades of TCI, as well as TCI of high purity have been used in the various assays. Technical, reagent and analytical grades contained a variety of additives as stabilizers (see list, Chapter 3) including the epoxide epichlorohydrin (1, chloro, 2,3-epoxypropane) and 1,2-epoxybutane, both monofunctional alkylating agents that are known mutagens (Fishbein, 1979; Table 7-1). Epichlorohydrin has also been demonstrated to be carcinogenic (Fishbein, 1979; Laskin et al., 1980). The technical grades of TCI commonly used in the bioassays contained 0.05 percent to 0.54 percent (w/w) of these epoxides (Henschler et al., 1977; Loprieno et al., 1979; Margard, 1978; NCI, 1976; Bell et al., 1978). The presence of these epoxides and other stabilizers has led to controversy

Table 7-1. CHRONOLOGY OF MUTAGENIC TESTS OF TCI*

Study	Test System	TCI chemical purity	Result
Greim et al., 1975	*E. coli*, K_{12} strain; activation	Purified	+
Ismailov and Ryskal, 1976	*D. melanogaster*	Technical grade	+
Fahrig, 1977	Mouse; somatic mutation test (spot test)	Purified	+
Henschler et al., 1977	*S. typhimurium* TA 100; activation	Technical grade Epichlorohydrin 1,2-epoxybutane	+ + +
Shahin and Von Borstel, 1977	*S. cerevisiae*, 185-14C haplid strain; activation	Purity unknown 10 & 20 µl/ml 0.5 µl/ml	 + -
Simmon et al., 1977	*S. typhimurium*, TA 100; activation	Purified vapor	+
Bronzetti et al., 1978	*S. cerevisiae*, strains D4 & D7 host-mediated assay, CD-1 mice; activation	Purified	+
Margard, 1978	*S. typhimurium* TA 1535, TA 1537 TA 1538, TA 98, TA 100; activation	Technical grade Purified	+ -
Price et al., 1978	*In vitro* Fischer rat embryo cell transformation test	Purified	+
Waskell, 1978	*S. typhimurium*, TA 100, TA 98 TA 1535, TS24recA, TA 2322 pol A, TA 1950 uvr B; activation	Purified Chloral hydrate TCA Trichloroethanol	- + - -

Table 7-1 (continued).

Study	Test System	TCI chemical purity	Result
Schairer et al., 1978	*Trandescantia*, clone 4430	Purity unknown (technical grade)	+
Konietzko et al., 1978	Human (hypodiploid cells) *in vivo*	Technical grade	+
Styles, 1979	*In vitro* baby Syrian hamster kidney (BHK-211Cl 13) cells	Purity not reported	+
Baden et al., 1979	*S. typhimurium*, TA 100, TA 1535 activation	Purified vapor liquid	 + -
Bartsch et al., 1979	*S. typhimurium*, TA 100, TA 98 activation	Purified	+
Loprieno et al., 1979	*S. pombe* - host-mediated assay B6C3F1 mice	Technical grade purified epichlorohydrin	+** - +
Mondino, 1979	*S. pombe* - host-mediated assay B6C3F1 mice	Purified	-
White et al., 1979	Chinese hamster ovary cells; sister chromatid exchanges; activation	Anesthetic grade vapor	-
Abrahamson and Valencia, 1980	*D. melanogaster* SL recessive lethal test	Technical grade aqueous sol. fed injected	 - -
Callen et al., 1980	*S. cerevisiae*, D7: gene conversion, mitotic recombination reverse mutation; *S. cerevisiae*, D4: gene conversion	Purified	+ +
Slacik-Erben et al., 1980	Mouse; dominant lethal mutation test	Purified	-

*See Appendix A for detailed information provided by the Reproductive Effects Assessment Group on doses used, responses observed, and comments on strengths and limitations.

**Reanalysis of the data suggest that the results were not statistically significant.

over interpretation of positive test results with assays of technical and other impure grades of TCI.

7.1 MUTAGENICITY

Trichloroethylene has been tested for its ability to cause gene mutations in bacteria, yeast, *Drosophila*, mice, and *Tradescentia*. It has been tested for its ability to cause primary DNA damage using the SCE technique and it has been studied for its ability to cause chromosome damage in humans. Additionally, tests have been performed addressing its effects on mitosis and its ability to reach the gonads of an intact mammal. Because TCI is highly volatile and not very soluble, it has been difficult to adequately test this material. Several investigators reporting negative results possibly failed to take these properties into account and, therefore, the concern is raised that the target cells were not adequately exposed. Another factor which makes it difficult to assess the significance of the reported results is that TCI is unstable and stabilizers must be added to it to prevent its decomposition. In the past, compounds known to be potent mutagens, such as epichlorohydrin, have been used as stabilizers. Therefore, positive results reported in papers not specifying the purity of the tested material and its comtaminants must be viewed with caution. These factors have rendered many of the reports concerning an assessment of the genotoxicity of TCI inappropriate. Several reports have indicated weak but positive responses in tests conducted with highly purified samples of TCI using bacteria, yeast, and more importantly intact mice. Thus, based on the weight of evidence available at this time, TCI is evaluated as being a weak mutagen. Since the metabolism and pharmacokinetics of this compound in humans results in metabolic products which can interact with DNA (e.g., chloral hydrate) as is the case for bacteria, yeast, and mice, it is concluded that it will likely cause mutagenic effects in humans as well, if it reaches DNA within exposed cells. Furthermore, if the compound or an active metabolite reaches the gonads, as is the case for mice, it may result in the production of heritable mutations. (See evaluation of mutagenicity reports presented in Section IV of Appendix, CAG report, for details of studies and a discussion and interpretation of the strengths and limitations of the data.)

7.2 CELL TRANSFORMATION

Price et al. (1978) reported the transformation of Fischer rat embryo (F1706, subculture 108) cells exposed to purified ICI. Also, subcutaneous injection of cells treated with ICI five subcultures earlier produced local fibrosarcomas in 13/13 and 12/12 newborn Fischer 344 rats within 48 to 55 days following treatment. However, the transformation of F1706 cells observed by Price et al. was not nearly as great as that produced by the positive control agent (3-methylcholanthrene).

In a second cell biotransformation assay, Styles (1979) exposed baby Syrian hamster kidney (BHK-211Cl 13) cells to unstabilized ICI (purity not reported) with and without metabolic activation provided by rat liver microsomes. Dose levels high enough to produce toxicity did not induce transformation. (See Appendix for detailed discussion and evaluation of these two cell transformation studies.)

7.3 ANIMAL BIOASSAYS

Evaluations of the animal bioassays for carcinogenicity of ICI are confounded by the familiar problem of purity of the ICI used, as well as by species difference in response, dosage and route, and adequacy of experimental design and execution. Table 7-2 provides a chronology and summary of tests performed to date. Some general observations can be made from Table 7-2. Assays have been carried out with both technical grade ICI (containing epichlorohydrin and epoxybutane) and purified chemical on several species (rats,

Table 7-2. TCI CARCINOGENICITY BIOASSAYS IN ANIMALS

Study	TCI chemical purity	Species	Dose levels, route	Results
NCI, 1976	Technical grade	Mice, B6C3F1 Males Females	1169, 2339 mg/kg/day 869, 1739 mg/kg/day gavage, 78 wk	Treatment-related liver hepatocellular carcinomas males and females
		Rats O-M	549, 1097 mg/kg gavage, 88 wk	Negative
Bell et al. (MCA), 1978	Technical grade	Mice B6C3F1	100, 300, 600 ppm inhalation, 24 mo	Treatment-related liver hepatocellular carcinomas males and females
		Rats CR	100, 300, 600 ppm inhalation, 24 mp	Negative
Maltoni, 1979	Purified	Rats S-D	250, 50 mg/kg gavage, 52 wk	Negative
Henschler et al., 1980	Purified	Mice Han:NMRI	100, 500 ppm inhalation, 78 wk	Increased incidence (females) malignant lymphomas
		Rats Han:Wist	100, 500 ppm inhalation, 78 wk	Negative
		Hamsters Syrian	100, 500 ppm inhalation, 78 wk	Negative
Van Duuren et al., 1979 Demopoulous et al., 1980	Purified	Swiss mice ICR/Ha Female	1 mg, 3x/wk, 581 d topical dermal	Negative

Table 7-2 (continued).

Study	TCI chemical purity	Species	Dose levels, route	Results
		Female	1 mg, 3x/wk, 14 d 2.5 µg phorbol myristic acetate, topical dermal 452 d	Negative
		Female Female Male	0.5 mg SC/wk, 622d 0.5 mg, once wk gavage, 622 d	Negative
		Female	1 mg TCI epoxide, 3x/wk, 2.5 µg phorbol myristic acetate, topical dermal, 452 d	Negative
		Female	2.5 mg TCI epoxide 3x/wk, dermal for 526 d	Negative
		Female	0.5 mg TCI epoxide, once wk, SC, 547 d	Negative
NCI (see Appendix)	Purified	Mice, B6C3F1 Rats	Gavage, 104 wk	In progress, completion date 1981.
		Osborne-Mendel Fisher 344, Marshall 540, August 28807 ACI	Gavage, 104 wk	
Maltoni (see Appendix)	Purified	Mice, B6C3F1 Swiss albino	Inhalation	In progress, completion date 1981.
		Rats Sprague-Dawley		

mice, hamsters) and several strains of these species. Only mice appear to be susceptible and develop tumors. The results of the NCI (1976) and MCA (Bell et al., 1978) studies indicate that B6C3E1 mice administered technical grade ICI by gavage and inhalation, respectively, developed a treatment-dependent incidence of hepatocellular carcinomas in both males and females (males more susceptible), while in Henschler's study, female (but not male) Han:NMRI mice administered purified ICI (free of epoxides) by inhalation developed a treatment-related incidence of malignant lymphomas. Van Duuren's study with epoxide-free ICI in male and female Han-ICR Swiss mice found no evidence that purified ICI induces cancer when tested by repeated skin application, with and without a promoter, by subcutaneous injection or by intragastric feeding. ICI epoxide was also negative in this study when tested by dermal application with a promoter and negative in another such study without a promoter.

The bioassay evidence that demonstrates a possible carcinogenic potential of ICI in mice (but not rats) is both strengthened and weakened by a closer examination of the individual assays.

7.3.1 NCI (1976)

Commercial (technical grade, 99.0 percent pure, w/w) ICI was tested in this study. Henschler et al. (1977) have suggested that the carcinogenic effects observed in this study were primarily due to the monofunctional alkylating agents epichlorohydrin (0.09 percent) and 1,2-epoxybutane (0.19 percent) added to ICI as stabilizers. Mutagenic analysis by these authors (Henschler et al., 1977), using IA 100 strain *Salmonella* in the Ames test, demonstrated that epichlorohydrin and 1,2-epoxybutane were potent mutagens, whereas purified epoxide-free ICI was not mutagenic. Commercial grade ICI was weakly mutagenic with metabolic activation.

> Male and female rats (Obsorne-Mendel) and mice (B6C3E1) were given ICI in corn oil by gastric intubation once daily 5

> days/week for 78 weeks. Two dose levels were studied. After 90 weeks (mice) and 110 weeks (rats), the surviving animals were sacrificed and extensively examined for gross and microscopic lesions. A total of 480 animals were studied consisting of groups of 50 TCI-exposed animals and 20 controls.

The results indicated a significant treatment-related increase in the spontaneous rate of hepatocellular carcinoma occurrence in the mice. The increase in the frequency of liver carcinomas in these animals was greater for the males than for the females. The first tumor appeared earlier in male mice treated with the higher dose levels than in the untreated controls. The results are summarized in Table 7-3. No increased frequency in the occurrence of hepatocellular carcinoma or other tumor was observed in the rats. Survival of both TCI-exposed and control rats was poor. Approximately 82 percent of the male controls and 60 percent of the female controls died before the termination of the experiment. The higher mortality rate among the rats significantly detracted from the usefulness of the assay in determining the carcinogenic potential of TCI in this species. This conclusion is supported by the finding that the rats also responded poorly to carbon tetrachloride, the positive control used in the assay.

Table 7-3. INCIDENCE OF HEPATOCARCINOMA IN B6C3F1 MICE EXPOSED TO TCI (NCI)

Males		
matched control	1/20 (5%)	
low dose (1169 mg/kg/day)	26/50 (52%)	$p<0.004$
high dose (2239 mg/kg/day)	31/48 (64%)	$p<0.001$
Females		
matched control	0/20 (0%)	
low dose (869 mg/kg/day)	4/50 (8%)	$p<0.09$
high dose (1739 mg/kg/day)	11/47 (23%)	$p<0.008$

There are several limitations in the NCI bioassay that must be considered in evaluation of the results as the ultimate test on the carcinogenic potential of ICI. In addition to ICI-exposed animal groups, animals which were being exposed to other volatile chemicals were housed in the same room. It is possible that the ICI-exposed animals were also being subjected to low level exposure to several other chemicals. The interaction potentiation of volatile compounds in the development of tumors is possible. Several expert committees have recommended that exposure of laboratory animals to a test chemical begin prior to conception, continue during pregnancy, with exposure of the offspring for life (FDA, 1971; Tomatis, 1974; Canada, 1973; Page and Arthur, 1978). Although in this bioassay exposure to ICI began just after weaning, the animals were still very young. Drug metabolizing systems are not well developed in younger animals nor are tissue and organ barriers. Thus these animals may have been more or less susceptible to the test compound. Furthermore, the studies were terminated after 110 weeks. Since 70 to 80 percent of the mice were surviving at that time, it is possible that late developing tumors might have been found if the experiment had been extended for a longer observation period. Another weakness in the design of the study was that exposure levels may have been too high, particularly in the rat study. Although these high doses increase the probability of a tumorigenic response by the test system, they may introduce toxic conditions that interfere with survival and/or the carcinogenic processes.

7.3.2 MCA (1978)

This assay was conducted at IBI Lab. Inc. (Chicago) from 1975-1977 for the Manufacturing Chemists Association who audited the study. The ICI (technical grade) tested in the study was similar in composition (ICI, 99.0 percent;

1,2-epoxybutane, 0.25 percent; epichlorohydrin, 0.15 percent) to that tested in the NCI assay. The audit findings were reported by Bell et al., 1978.

> Male and female rats (Charles River) and mice (B6C3F1) were given TCI by inhalation, 6 hr/day, 5 days/wk for 24 months at 100, 300 and 600 ppm (0.55, 1.65, and 3.30 mg/l air). The number of animals studied was 140 mice and 120 rats of each sex/dose group for each of the three treatment groups and an untreated control group. Surviving animals were sacrificed on termination of treatment. Forty mice/sex and 30 rats/sex scheduled for interim sacrifice to evaluate hematology and clinical chemistry were not given a pathologic examination.

The execution of the assay was greatly flawed. Control mice of both sexes were replaced during the study soon after it began, although the replacement mice were from the same supplier. During the first 4 months, the vapor doses for all animals fluctuated greatly with consequent dose level overlaps; the dose monitoring data covered only the initial 6 months; and data for the final 18 months of the study were not reported (Bell et al., 1978). Furthermore, the pathology was inaccurately recorded. The results of this assay in mice are summarized below. A carcinogenic effect of TCI in rats was not found. Only the liver was examined.

Table 7-4. INCIDENCE OF HEPATOCARCINOMA IN B6C3F1 MICE EXPOSED TO TCI (MCA)

Males	No.*		
controls	99	18/99 (18.2%)	
dose - 100 ppm	95	28/100 (29.5%)	$p<0.05$
dose - 300 ppm	100	31/100 (31.0%)	$p<0.03$
dose - 600 ppm	97	43/100 (44.3%)	$p<0.00006$
Females			
controls	99	6/99 (6.0%)	
dose - 100 ppm	100	4/100 (4.0%)	
dose - 300 ppm	94	9/94 (9.6%)	$p = 0.26$
dose - 600 ppm	99	13/99 (13.1%)	$p = 0.07$

*For each group, 140 mice were put on study; 40 in each dose group were interim sacrificed.

The data indicate a treatment-related, statistically significant increase in the incidence of hepatocellular carcinomas in male mice ($p<0.05$), and an increase (although not statistically significant) in female mice at the two higher dose levels.

The results of this assay of TCI (technical grade) by inhalation are in apparent general agreement with those of the NCI assay of TCI (technical grade) by gavage. However, serious reservations about the validity of the IBT assay are engendered because of the faulty execution of the assay. In addition, a higher incidence of hepatocarcinoma appeared in the male controls (18.2 percent) compared with 5 percent in the NCI study (Table 7-3), although this is still in the range of historical controls.

7.3.3 Maltoni (1979)

Maltoni (1979) summarized the results of an assay of purified TCI in Sprague-Dawley rats. The TCI was epoxide-free, at least 99.9 percent pure, and contained 0.001 percent diisopropylamine as a stabilizer.

> Male and female rats (Sprague-Dawley, 13 wk old) were given TCI in corn oil by gastric intubation once daily, 5d/wk for 52 wk. Two dose levels were studied, 50 and 250 mg/kg. A total of 150 rats were studied with 30 animals for each dose group, and a control group given olive oil. The animals were sacrificed at 140 wk for necropsy examination and histopathologic examination.

Purified TCI was without carcinogenic effect in this rat assay. This finding is in agreement with the NCI gavage study using technical grade TCI and Osborne-Mendel strain of rats. However, the TCI dose levels tested were considerably lower than in the NCI study, and the rats were exposed for a shorter time, 52 wk, with treatment initiated at 13 wk of age.

7.3.4 Henschler et al., (1980)

Henschler et al. (1980) reported an inhalation assay of the carcinogenic potential of purified epoxide-free TCI in mice, rats and hamsters. The TCI was at least 99.9 percent pure and contained 0.0015 percent triethanolamine added as a stabilizer.

> Male and female rats (Han:Wist), mice (Han:NMRI) and Syrian hamsters were administered TCI by inhalation 6 hr/d, 5d/wk for 18 months at two dose levels, 100 and 500 ppm (0.55 mg/l, and 2.8 mg/l air). Thirty animals of each species were studied for each sex/dose group, for each of the two treatments, and for an untreated control group. Surviving mice and hamsters were sacrificed at 30 months, and rats at 36 months, for necropsy and histopathologic examination. At 30 months, the percentage of mice and hamsters surviving were: mice, control 25%, 100 ppm 7%, 500 ppm 3%; hamsters, control 13%, 100 ppm 7%, 500 ppm 27%. At 36 months, the percentage of rats surviving were: control m. 47%, f. 17%; 100 ppm m. 23%, f. 13%; 500 ppm m. 37%, f. 17%.

This study showed no carcinogenic effect of pure TCI in rats, hamsters or male mice. Male mice were the most susceptible to hepatocellular carcinoma in the NCI and MCA studies.

Female mice did show a statistically significant increased incidence of malignant lymphomas. The results are summarized as follows:

Table 7-5. INCIDENCE OF MALIGNANT LYMPHOMA IN FEMALE HAN:NMRI MICE EXPOSED TO TCI.

Control group	9/29 (31.0%)
100 ppm inhalation	17/30 (56.7%) $p = 0.04$
500 ppm inhalation	18/30 (60.0%) $p = 0.01$

Henschler et al. (1980) concluded that the increased incidence of malignant lymphomas in female mice could have been due to an occurrence of immunosuppression which enhanced the susceptibility of the animals to tumor induction by specific, mostly inborn, viruses. The high incidence (31 percent) in the control group provides support for this conclusion. Sanders et al. (1980) and Tucker et al. (1980) in preliminary reports have observed a dose-related reduction in the immune response of CD-1 mice given gavage doses of 24 or 240 mg/kg of TCI for

14 days; therefore, the incidence of enhancement of the lymphoma incidence in the treated female mice possibly could have been due to an immunosuppressive action of TCI.

7.3.5 Van Duuren et al., (1979)

Van Duuren et al. (1979) reported a series of assays of purified epoxide-free TCI in ICR/Ha Swiss mice. The mice were 6 to 8 weeks old at the onset of testing. The assays were as follows:

> Dermal: (a) female mice received topical application on shaved skin of 1 mg TCI in acetone, 3x weekly for 581 days. A vehicle-controlled group of 30 female mice, and an untreated control group of 100 female mice were included.
>
> (b) 30 female mice received topical application on shaved skin of 1.0 mg TCI in acetone followed 14 days later by application of 2.5 μg phorbol myristic acetate in acetone 3x weekly for 452 days. The control groups included: 30 female mice treated with vehicle alone, 105 female mice treated with phorbol myristic acetate alone, and 100 female mice untreated.
>
> (c) 30 female mice received topical application on shaved skin of 1.0 mg TCI epoxide in acetone followed 14 days later by applications of 2.5 μg phorbol myristic acetate in acetone 3x weekly for 452 days. The control groups were as in (b).
>
> Subcutaneous: 30 female mice were given subcutaneous injections of 0.5 mg TCI in trioctanoin weekly for 622 days. The control groups were: 30 female mice given vehicle alone, and 100 female mice untreated.
>
> Gavage: 30 male and 30 female mice were given, by gastric intubation, 0.5 mg TCI in trioctanoin weekly for 622 days. The control groups were: 30 mice in male and female groups given vehicle alone, and 100 mice in male and female groups untreated. At sacrifice, the animals were examined at necropsy and histopathology was carried out on skin, liver, stomach and kidney.

These assays were negative and provided no evidence of a carcinogenic effect of TCI. However, use of maximum tolerated doses in the skin treatment is not evident, and oral and subcutaneous doses were administered only once per week. Moreover, only liver, kidney, stomach, and/or skin were evaluated histologically.

Demopoulous et al. (1980) reported on prepublication data from dermal and subcutaneous assay injections of TCI epoxide conducted in Van Duuren's laboratory on female ICR/Ha mice.

> Dermal: 30 mice were treated with topical applications of 2.5 mg TCI epoxide in acetone 3x weekly for 526 days. Control groups consisted of 30 vehicle treated mice and 100 untreated mice. A positive control group was treated with topical applications of 3.3 mg d,l-diepoxybutane, 3x weekly for 462 days.
>
> Subcutaneous: 30 mice were given, subcutaneously, 0.5 mg TCI epoxide in tricaprylin, once weekly for 475 days. Control groups consisted of 30 mice given the vehicle and 100 untreated mice. A positive control group was treated with 0.33 mg d,l-diepoxybutane, once weekly for 475 days.

TCI epoxide was without carcinogenic effect at local sites of administration in these two assays. However, d,i,-diepoxybutane was strongly positive producing squamous cell carcinomas and sarcomas at local sites of administration. Data on tumor formation at distant sites were not available.

7.4 EPIDEMIOLOGIC STUDIES

At present there are only a few epidemiologic studies which have investigated the carcinogenic effects in a cohort population exposed to TCI. They all suffer from design inadequacies leading to inconclusive outcomes due to one or more of the following factors: (1) small size of the cohort population, (2) relatively young age of the cohort, (3) relatively few death occurrences, and (4) a short or no follow-up period. In addition, the cohort population might also have been exposed to other solvents or other cancer-risk factors, e.g., cigarette smoking, of which the interactive effects are difficult to assess.

Blair et al. (1979) in a retrospective study examined the relative frequency of specific causes of death of 330 members of a laundry and dry-cleaning workers union utilizing union mortality records for the period 1957-1977. In this small group a statistically significant increased proportion of cancer deaths was noted (p<0.5). The significant increases were lung and cervical cancer, with slight excesses of leukemia and liver cancer. However, the cohort was also exposed to several other solvents used in the laundry and dry-cleaning industry, including the potential carcinogens tetrachloroethylene, carbon tetrachloride and benzene. This study is being continued and extended to a prospective study of 2 to 5 thousand workers from the union, but it is unlikely that sufficient information will be obtained to attribute any excess cancer among dry-cleaning workers solely to TCE

Axelson et al. (1978) have published a retrospective study of 518 male Swedish workers with a history of exposure to TCE prior to 1970 and having subcohorts with a 10-year latency period. Since 1950, Swedish workers exposed to TCE have had measurements of their exposure to TCE recorded by measuring the trichloroacetic acid (TCA) metabolite in their urine (see Chapter 4.5, Measures of exposure and body burden). The cohort was divided into high- and low-exposure groups based on these records. The high dose group probably was exposed to the current Swedish air standard of 30 ppm (100 mg TCA/l urine) Neither group showed any significant excess of cancer mortality. However, there were several possible limitations to this study (see Appendix A).

A similar study of Finnish workers (1,868) exposed to TCE from 1965 is ongoing. A preliminary report (Tola, 1978) showed no excess of cancer mortality. The cancer incidence, 10/47 deaths from all causes, showed no predominant type.

Two retrospective studies have been performed recently using hospital medical records and death certificates for people employed in the dry cleaning and laundry industries. In the study by Lin and Kessler (1981), medical records obtained from 115 hospitals were used to identify both individuals who had a specific type of cancer and a group of cancer free matched controls.

One of the associations observed was a relationship between pancreatic cancer and exposure to dry cleaning chemicals and gasoline fumes. Although this suggests the need for further epidemiology studies as to the hazards of the dry cleaning industry, the lack of any information on the extent or type of chemical exposure does not allow an association to be made between TCI exposure and pancreatic cancer. In the second study, Katz and Jowett (1981) examined the death certificates of 671 female workers in the laundry and dry cleaning industry in order to determine the cause of death. The cause-specific proportionate mortality ratios (PMRs) were calculated for 25 major causes of death, using female workers in the categories of service and operative workers for comparison. An elevated risk of genital (unspecified) and kidney cancer was observed along with an elevated risk of diabetes mellitus. Again the lack of data on the extent of exposure and the types of chemicals to which the workers were exposed makes interpretation very speculative. Both of these studies indicate that further investigation of the laundry and dry cleaning industries are needed.

Konietzko et al. (1978) reported a study that suggests TCI may possibly cause mutagenic or genetic effects in humans. The investigators studied a small group (28) of workers in a degreasing plant employing TCI. An increased proportion of hypodiploid cells was found among degreasers who had greater numbers of peak exposures to TCI. Although the details of the exposure histories are scanty and the cohort small, the find is statistically significant.

7.5 PERSPECTIVE

It is evident from this review of the data that no one bioassay can be singled out and accepted as definitive evidence of the carcinogenic potential of TCI. Each bioassay reported, whether an in vitro mutagenic test or an in vivo animal lifetime carcinogenesis study, had its own limitations of one or more factors: chemical purity of TCI tested, appropriateness of dose and/or mode of administration and duration, species used, and species differences in sensitivity and reponse, metabolism and pharmacokinetic considerations. The few epidemiologic studies available are inconclusive and do not contribute significantly either for or against a carcinogenic potential for TCI.

A summary of the evidence of a carcinogenic potential for TCI is as follows: (1) technical grade TCI was shown to be positive by different investigators in mutagenic tests with S. typhimurium TA 100 and TA 1535 strains, with S. cerevisiae 185-14C haploid strain and with S. pombe host-mediated assay. Analytic and reagent grade TCI was shown to be positive by different investigators in mutagenic tests with S. typhimurium TA 100 and TA 1535 strain, with E. coli K_{12} strain and with S. cerevisiae strains D4 and D7. Purified analytical or reagent grade TCI was also positive in the mouse somatic mutation test (spot test) and in an in vitro cell transformation test with a Fischer F1706 rat embryo cell line. In these assays TCI required metabolic activation to produce a mutagenic response, and TCI is thought to be metabolized in mammalian systems to a reactive epoxide intermediate as well as to other metabolites, such as chloral hydrate, which was shown to be weakly mutagenic in a S. typhimurium TA 100 assay. (2) Technical grade TCI was shown to induce hepatocellular carcinomas in male and female B6C3F1 mice in two different lifetime studies by gavage and inhalation. However, technical grade TCI was not shown to be carcinogenic in two different rat strains. Purified TCI increased the lymphoma incidence in female mice when administered by inhalation, although other animal assays using pure epoxide-free TCI did not indicate cancer-causing activity.

The evidence for a carcinogenic potential for highly pure TCI is less convincing. Although, as previously pointed out, purified analytical or reagent grade ICI was positive in test conducted with S. typhimurium IA 100 and IA 1535, with E. coli strain K_{12}, with S. cerevisiae strains D_4 and D_7, in the mouse somatic mutation test (spot test), and in an *in vitro* cell transformation test, purified epoxide-free ICI was found to be without carcinogenic activity in 3 different lifetime studies involving two strains of rats (gavage and inhalation), Syrian hamsters (inhalation) and 2 strains of male and female mice (gavage, subcutaneous and dermal). ICI epoxide was also without activity with dermal application in female Swiss mice with and without initiation-promotion. However, purified epoxide-free ICI, in a lifetime inhalation study with female Han:NMRI mice, was found to be associated with an increased incidence of malignant lymphomas. The high spontaneous incidence of lymphoma in this mouse strain, and evidence that ICI may possess immunosuppressive activity, suggest that the susceptibility of these mice to viral tumor induction was increased by ICI.

In summary, the weight of the evidence indicates that technical grade ICI possesses mutagenic and carcinogenic activity. In addition, evidence exists that pure ICI is a potential carcinogen although the data are less sufficient. The question remains of whether the carcinogenic potential of technical ICI is due to ICI per se, to the contaminating epoxides and other impurities, or to a mutual potentiation. Arguments have been made that the amount of epoxides present in technical grade ICI was insufficient to produce positive test reponses, that these epoxides are direct-acting mutagens and carcinogens and do not require metabolic activation as does ICI, and that these epoxides produce in animal bioassays a different tumor profile than technical grade ICI. The carcinogenicity of pure ICI devoid of epoxides is currently in retest by a lifetime NCI gavage study in B6C3F1 mice and several rat strains, and by Maltoni and associates in a lifetime inhalation study in mice and rats (Table 7-2). Hopefully, these ongoing studies will provide data leading to a more definite conclusion.

7.5 REFERENCES

Abrahamson, S., and R. Valencia. Evaluation of substances of interest for genetic damage using Drosophilamelanogaster. Final sex-linked recessive lethal test report to FDA on 13 compounds. FDA Contract No. 233-77-2119, 1980.

Axelson, O., K. Anderson, C. Hogstedt, B. Holmberg, G. Molina, and A. de Verdier. A cohort study on trichloroethylene exposure and cancer mortality. J. Occup. Med. 20:194-196, 1978.

Baden, J.M., M. Kelley, R.I. Mayze, and V.F. Simmon. Mutagenicity of inhalation anesthetics: Trichloroethylene, divinyl ether, nitrous oxide and cyclopropane. Br. J. Anaesth. 51:417-421, 1979.

Banerjee, S., and B.L. Van Duuren. Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA in vitro. Cancer Res. 38:776-780, 1978.

Bartsch, H., C. Malaveille, A. Barkin and G. Planche. Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes and dichlorobutanes produced by rodent or human liver tissues. Arch. Toxicol. 41:249-278, 1979.

Bell, Z.G., K.J. Olson, and T.J. Benya. Final report of audit findings of the Manufacturing Chemists Association (MCA): Administered trichloroethylene (TCE) chronic inhalation study at Industrial Bio-test Laboratories, Inc., Decatur, Illinois. Unpublished. 1978.

Blair, A., P. Decoufle, and D. Grauman. Causes of death among laundry and dry cleaning workers. Am. J. Publ. Hlth. 69:508-511, 1979.

Bolt, H.M., A. Buchter, L. Wolowski, D.L. Gil, and W. Bolt. Incubation of ^{14}C-trichloroethylene vapor with rat liver microsomes: uptake of radioactivity and covalent binding of metabolites. Inter. Arch. Occup. Environ. Hlth. 39:103-111, 1977.

Bonse, G., T. Urban, D. Reichert, and D. Henschler. chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem. Pharmacol. 24:1829-1834, 1975.

Bronzetti, G., E. Zeiger, and D. Frezza. Genetic activity of trichloroethylene in yeast. J. Environ. Pathol. and Toxicol. 1:411-418, 1978.

Callen, D. F., C. R. Wolf, and R. M. Philpot. Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccaromyces cerevisiae. Mutat. Res. 77:55-63, 1980.

Canada, Ministry of Health and Welfare. The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity, 1973.

Carcinogen Assessment Group Report on Trichloroethylene External Review Draft. April, 1981.

Creech, J.L., Jr., and M.N. Johnson. Angiosarcoma of the liver in the manufacture of polyvinyl chloride. J. Occup. Med. 16:150-151, 1974.

Demopoulos, H.B., B. Wagner, and J. Cimino. An academic review of the hazards posed by trichloroethylene. Unpublished document presented at EPA Science Advisory Board meeting on Air Carcinogens, Washington, DC, Sept. 3-4, 1980.

Fahrig, R. The mammalian spot test (Fell fleckentest) with mice. Arch. Toxicol. 38:87-98, 1977.

Fishbein, L. Potential halogenated industrial carcinogenic and mutagenic chemicals. I. Halogenated unsaturated hydrocarbon. Sci. Total Environ. 11:111-161, 1979.

Food and Drug Administration Advisory Protocols for Safety Evaluation. Panel on Carcinogenesis Report on Cancer Testing in the Safety of Food Additives and Pesticides. Toxicol. Appl. Pharmacol. 20:419-438, 1971.

Greim, H., G. Bonse, Z. Radwan, D. Reichert, and D. Henschler. Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochem. Pharmacol. 24:2012-2017, 1975.

Gu, Z. W., B. Sele, P. Jalbert, M. Vincent, F. Vincent, C. Marka, D. Chmara and J. Faure. Induction d'echanges entre les chromatides soeurs (SCE) par le trichloroéthylène et ses métabolites. Toxicologie Métabolique. 3:63-67, 1981.

Henschler, D., and G. Bonse. Metabolic activation of chlorinted ethylenes. Dependence of mutagenic effect on electrophilic reactivity of the metabolically formed epoxides. Arch. Toxicol. 39:7-12, 1977.

Henschler, D., E. Eder, T. Neudecker, and M. Metzler. Carcinogenicity of trichloroethylene: Fact or artifact? Arch. Toxicol. 37:233-236, 1977.

Henschler, D., W. Romen, H.M. Elasser, D. Reichert, E. Eder, and Z. Radwan. Carcinogenicity study of trichloroethylene by long-term inhalation in the animal species. Arch. Toxicol. 43:237-248, 1980.

International Agency for Research on Cancer (IARC). Trichloroethylene. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. 20:545-572, 1979.

Ismailov, A.S., and Ryskal, G.V. Mutagenic effect of TCE in the offspring of the fly *Drosophila melanogaster*. Biol. Nauk. Iss. 1:6-10, 1976.

Katz, R. M., and D. Jowett. Female laundry and dry cleaning workers in Wisconsin: A mortality analysis. A.J.P.H. 71:305-307, 1981.

Konietzko, H., W. Haberlandt, H. Heilbronner, G. Reill, and H. Weichardt. Chromosome studies on trichloroethylene workers. Arch. Toxicol. 40:201-206, 1978.

Laib, R.J., G. Stockle, H.M. Bolt, and W. Kung. Vinyl chloride and trichloroethylene: Comparison of alkylating effects of metabolies and induction of preneoplastic enzyme deficiencies in rats livers. J. Can. Res. Clin. Oncol. *94*:139-147, 1979.

Laskin, S., A.R. Sellakumar, M. Kuschner, S. LeMendola, N. Nelson, G M. Rusch, G.V. Katz, N.C. Dulak, and R.E. Albert. Inhalation carcinogenicity of epichlorohydrin in noninbred Sprague-Dawley rats. J. Natl. Cancer Inst. *65*:751-755, 1980.

Lin, R. S., and I. I. Kessler. A multifactorial model for pancreatic cancer in man. J.A.M.A. *245*:147-152, 1981.

Loprieno, N., R. Barale, A.M. Rossi, S. Fumerjo, G. Meriggi, A. Mondino and S. Silvestri. *In vivo* mutagenicity studies with trichloroethylene and other solvents (preliminary results). Unpublished. 1979.

Maltoni, C. Results of long-term carcinogenicity bioassays of trichloroethylene experiments by oral administration on Sprague-Dawley rats. In press, 1979.

Maltoni, C. and G. Lefemine. Carcinogenicity bioassays of vinyl chloride: current results. Ann. NY Acad. Sci. *246*:195-218, 1975.

Margard, W. Summary report on *in vivo* bioassay of chlorinated hydrocarbon solvents. Unpublished. 1978.

Mondino, A. Examination of the live mutant activity of the trichloroethylene compound with "*Schizosaccharomyces pombe*." Unpublished. 1979.

NCI. Carcinogenesis bioassay of trichloroethylene. National Cancer Institute Carcinogenesis Technical Report Series No. 2, HEW Publication No. NIH 76-802, February 1976. 197 pp.

Page, N. P., and J. L. Arthur. Special Occupational Hazard Review of Trichloroethylene. DHEW (NIOSH) Publication No. 78-130. 79 pp. 1978.

Price, P.J., C.M. Hassett, and J.I. Mansfield. Transforming activities of trichloroethylene proposed and industrial alternatives. *In Vitro* *14*:290-293, 1978.

Sanders, V.M., A.N. Tucker, P.H. Hallett, B. Kaufman, R.A. Carchman, and A.E. Munson. Immunotoxicology of trichloroethylene in the mouse. II. Effects on humoral and cell-mediated immunity. Unpublished. 1980.

Schairer, L. A., J. Van't Hof, C. G. Hayes, R. M. Burton, and F. J. deSerres. Measurement of biological activity of ambient air mixtures using a mobile laboratory for *in situ* exposures. Preliminary results from the *Tradescantia* plant test system. EPA Publ. 600/9-78-027: 421-440, 1978.

Shahin, M.M., and R.C. VonBorstel Mutagenic and lethal effects of benzene hexachloride, dibutyl phthalate and trichloroethylene in *Saccharomyces cerevisiae*. Mutat. Res. *48*:173-180, 1977.

Simmon, V.F., K. Kauhanen, and R.G. Tardiff. Mutagenic activity of chemicals identified in drinking water. Pages 249-258 in D. Scott, B.A. Bridges and F.H. Solves, eds. Progress in genetic toxicology. Elsevier/North Holland Biomedical Press, 1977.

Slacik-Erben, R., R. Roll, G. Franke, and H. Uehleke. Trichloroethylene vapours do not produce dominant lethal mutations in male mice. Arch. Toxicol. 45:37-44, 1980.

Styles, J. A. Cell transformation assays. In: Topics in Toxicology: Mutagenesis in Sub-mammalian Systems: Status and Significance. G. E. Paget, Ed. University Park Press Baltimore, 1979. pp. 147-164.

Tola, J. As cited by Page, N.P. and J.L. Arthur (eds.). In: Special Occupational Hazard Review of Trichloroethylene. 79 pp. DHEW NIOSH 78-130, 1978.

Tomatis, L. Report of Discussion Group No. 4: Inception and Duration of Tests. In: Carcinogenesis Testing of Chemicals, L. Golberg, (ed.), CRC Press, Cleveland, OH, pp. 23-27, 1974.

Tucker, A.N.M., V.M. Sanders, D.W. Barnes, T.J. Bradshaw, and A.E. Munson. Immunotoxicology of trichloroethylene in the mouse. I. General toxicology. Unpublished. 1980.

Uehleke, H., and S. Poplawski-Tabarelli. Irreversible binding of ^{14}C-labelled trichloroethylene to mice liver constituents in vivo and in vitro. Arch. Toxicol. 37:289-294, 1977.

Van Duuren, B.L., and S. Banerjee. Covalent interaction of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res. 36:2419-2422, 1976.

Van Duuren, B.L., B.M. Goldschmidt, G. Lowengart, A.C. Smith, S. Melchionne, I. Seldman, and D. Roth. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. J. Natl. Cancer Inst. 63:1433-1439, 1979.

Viola, P.L., A. Bigotti, and A Caputi. Oncogenic response of rat skin, lungs, and bones to vinyl chloride. Cancer Res. 31:516-522, 1971.

Waskell, L. A study of the mutagenicity of anesthetics and their metabolites. Mutat. Res. 57:141-153, 1978.

Waters, E.M., H.B. Gerstner, and J.E. Huff. Trichloroethylene. I. An overview. J. Toxicol. Environ. Hlth. 2:671-707, 1977.

Waxweiler, R.J., et al. Neoplastic risk among workers exposed to vinyl chloride. Ann. NY Acad. Sci. 271:40-48, 1976.

White, A. S., S. Takehisa, E. I Eger, II, S. Wolff, and W. C. Stevens. Sister-chromatid exchanges induced by inhaled anesthetics. Anesthesiology 50:426-430, 1979.

8. SUMMARY OF ADVERSE HEALTH EFFECTS AND LOWEST OBSERVED EFFECT LEVELS

8.1 SUMMARY OF TOXIC EFFECTS

Toxicological effects on the central nervous system (CNS) and cardiovascular system, skin, liver, and kidney have been attributed to TCI exposure. Effects on the CNS, principally depression, have been well documented. Trigeminal palsies and visual deterioration have also been reported. Dermatological problems including irritation, burns, and rashes result from skin contact with the chemical. Fatal hepatic failure and renal failure have been observed following use of TCI as an anesthetic. In addition, cardiovascular and respiratory effects with a number of deaths attributed to respiratory arrest, cardiac arrhythmias including ventricular fibrillation, and primary cardiac standstill have resulted from acute exposures. Dose-response relationships for adverse health effects associated with TCI have not been established in man or experimental animals. Toxic dose data are presented in Table 5-1.

Intolerance to alcohol is a well-characterized phenomenon that results from TCI exposure. This effect has been reported to occur at TCI levels as low as 5 ppm. Classically, TCI is recognized as being a CNS depressant, and evidence exists for more persistent CNS effects resulting from higher level exposure, or more importantly, from lower-level chronic exposure.

8.1.1 Animal Toxicity Studies Useful for Hazard Assessment. The preferred studies for hazard assessment are those which provide definite effect levels. Adverse effects are defined here as functional impairment and/or pathological lesions which may affect the performance of the whole organism or which reduce an organism's ability to respond to an additional challenge. Adverse effects which are not carcinogenic are assumed to be threshold phenomena. The threshold region of toxicity is estimated by evaluating four types of effect levels:

NOEL: No-Observed-Effect Level: That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

NOAEL: No-Observed-Adverse-Effect Level: That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse.

LOAEL: Lowest-Observed-Adverse-Effect Level: The lowest exposure level in a study or group of studies which produces statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

LOEL: Lowest-Observed-Effect Level: That exposure level in a study or group of studies which produces statistically significant increases in frequency or intensity of effects between the exposed population and its appropriate control.

FEL: Frank-Effect Level: That exposure level which produces unmistakable adverse effects, ranging from reversible histopathological damage to irreversable functional impairment or mortality, at a statistically significant increase in frequency or severity between an exposed population and its appropriate control.

Studies providing both a NOAEL and a LOAEL, therefore, are the most useful studies for hazard assessment.

8.2 INHALATION EXPOSURE

As a result of the volatility of TCI and its use patterns, the most common route of human exposure is inhalation. Psychomotor function and subjective responses which have been studied in short-term controlled human studies, as well as data from accidental exposure and epidemiological studies, indicate that nervous system function probably is affected by TCI concentrations ranging from 1 to 500 ppm. This evidence suggests that a threshold level for general CNS depressant activity or for acute effects of TCI is probably close to or below the established TLV and the OSHA standard of 535 mg/m^3 (100 ppm). Many other countries, especially several eastern European countries, have set much lower limits for occupational standards for TCI (Table 5-2). A few experimental human studies have been performed using single or repeated inhalation exposures to TCI. Although these studies provide precise information on exposure levels, they are limited by the small numbers of subjects employed in the studies and the restricted number of parameters that were assessed. Additional quantitative data regarding the acute effects of TCI can be obtained from experiments with animals and from the use of TCI as an anesthetic. Accounts of acute or chronic exposure of humans are compromised by uncertainties regarding the level and duration of exposure, and potential exposure to other chemicals including toxic stabilizers (epoxides) commonly added to TCI, and decomposition products. No chronic animal studies that adequately define no-effect or low-effect levels of inhalation exposure were available. Two subchronic animal inhalation studies can be used for this purpose.

8.2.1 Effects of Single or Acute Inhalation Exposure

A few controlled studies on the acute effects of TCI inhalation have been conducted with human subjects. These studies attempted to assess potential effects on the central nervous system (CNS). Short exposures (2 hours or less) to 100 ppm appeared to have no effect on subjects' psychomotor performance (Stopps and McLaughlin, 1967; Vernon and Ferguson, 1969), but longer exposures (two 4-hour exposures separated by a 1-1/2-hour break) to about the same concentration (110 ppm) produced statistically significant decreases in performance on tests of perception, manual dexterity, reaction time, and Wenchsler memory scale in the 6 male students tested (Salvini et al., 1971). Transient eye irritation and slight dizziness were reported by subjects during peak fluctuations of the exposure concentration to 130 ppm (Salvini et al., 1971).

The threshold for odor recognition was 21.4 ppm (Leonardos et al., 1969). Exposure to 1000 ppm for 2 hours decreased the fusion limit during measurements of optokinetic nystagmus in 12 subjects (Kylin et al., 1967).

Qualitative information on the effects of exposures to high TCI concentrations can be derived from reports of industrial accidents, abuse (sniffing) cases, and the use of TCI as an anesthetic. Cases of acute industrial overexposure have involved CNS and cardiac effects. The victims suffered headache, dizziness, tremors, sleepiness, lightheadedness, drowsiness, and sometimes nausea, vomiting, unconsciousness, and death (Stuber, 1931; Kleinfeld and Tabershaw, 1954). Some deaths were attributed to ventricular fibrillation (Kleinfeld and Tabershaw, 1954). Effects on the kidney were rare and no cases of liver injury were reported. Individuals who intentionally inhaled TCI for its intoxicating properties have suffered acute hepatic and renal damage (Baerg and Kimberg, 1970; Clearfield, 1970). Fatalities from TCI abuse were attributed to acute cardiac and respiratory failure. Patients anesthetized with TCI generally had no evidence of liver or kidney damage (Brittain, 1948; Defalque, 1961). Fatal acute hepatic failure occasionally occurred, but the affected patients usually had conditions such as malnutrition, toxemia and burns, or had received transfusions. Cardiac arrythmias have also been observed in patients anesthetized with TCI (Section 5.3.1.). Precise dose-response data for these effects are not available; when used medically as an anesthetic, TCI is administered in concentrations of 0.5 to 1 volume percent (5,000 to 10,00 ppm) (Section 4.1).

Because the human data are inadequate for defining a dose-response relationship, particularly at high levels of exposure, animal data will be used to aid in estimating levels of exposures that might result in impairment of psychomotor function, narcosis, organ damage, and death. Exposure of rats to 200 to 800 ppm TCI for 3 to 6 hours produced behavioral changes indicative of a loss of inhibition and, at the higher end of the range, slight decrements in swimming performance (Grandjean, 1960, 1963). Rats exposed to 1600 ppm for 6 hours had significant decrements in swimming performance and appeared to be fatigued (Grandjean, 1963). At a 5500 ppm exposure level, the anesthetic ET_{50} in mice was 46 minutes and the ET_{50} for hepatotoxicity was 400 minutes, very close to the LT_{50} of 585 minutes (Gehring, 1968). In other words, exposures of a duration sufficient to kill some of the mice were required in order to produce liver damage in a significant number of the survivors (Gehring, 1968). Higher levels of exposure, 12,000 to 15,000 ppm, produced narcosis in rats within minutes (Utesch et al., 1981). In a simulation of human solvent abuse, rats were exposed cyclically to 15,000 ppm in a 5 minutes off, 15 minutes on regimen for a total of 5 hours. (Loss of righting reflex occurred after 3 minutes, 37 seconds exposure to 15,000 ppm.) No elevation of SGOT or SGM occurred as a result of this regimen and no histopathological evidence of renal or hepatic damage was found in the treated animals (Utesch et al., 1981). The lack of liver or kidney damage in these controlled animal experiments contrasts with the case reports of solvent abusing mentioned previously. Solvent abusers, however, may have been using cleaning fluids and other mixtures containing TCI, rather than the pure chemical, and may have had predisposing conditions such as poor nutrition and abuse of other drugs. LC_{Lo} values for rats, mice, and cats were in the range of 3000 to 8000 ppm for 2 to 4-hour exposures (Table 5-1).

By compiling the available animal and human data, it is possible to establish a crude relationship between exposure and effects. This approach appears justified because the levels of exposure that produced slight behavioral effects and those that produced narcosis were similar in humans and animals. A summary of the simulated dose-response relationships is presented below:

Concentration	Response
21 ppm	odor recognition threshold
100-200 ppm	first signs of CNS depression, giddiness, slight eye irritation
1,000-2,000 ppm	impaired performance
3,000-5,000 ppm	narcosis with prolonged exposure; possibly life-threatening
5,000-10,000 ppm	anesthetic level; possibly life-threatening; possible cardiac arrythmias
>12,000 ppm	narcosis within minutes; life-threatening; possible liver or kidney damage

It should be evident that these estimates are at best rough approximations. Furthermore, experiments involving daily exposure of human subjects to low levels of TCI (100 to 200 ppm) for about 5 days have not indicated significant neurological or psychomotor impairment (Section 8.1.2).

8.2.2 Effects of Intermittent or Prolonged Inhalation Exposure

As was the case for single exposures, the few experimental studies of the effects on humans of repeated exposure to TCI were primarily concerned with potential CNS effects. Subjects exposed to 200 ppm TCI for 7 hours a day experienced mild fatigue and sleepiness after 4 to 5 days' exposure (Stewart et al., 1970). Some subjects reported eye irritation and subjective complaints such as headache and lightheadedness but these complaints occurred only on the first day. Half of the subjects said they had difficulty in performing a modified Romberg test. Scores on performance and neurological tests, however, were normal. Similarly, exposure of subjects to about 100 or 200 ppm, 4 hours a day for 6 days did not affect their performance on flicker fusion or two-point discrimination tests; the subjects complained of headaches (Nomiyama and Nomiyama, 1977). The lack of effect from these repeated exposures to 100 and 200 ppm TCI does not bear out the concern raised by the adverse effects on perception, memory, and performance noted after single exposures to 110 ppm by Salvini et al. (1971).

No adequate epidemiologic studies of the potential (noncarcinogenic) toxicity of TCI were available. Occupational exposure to TCI has been associated with CNS disorders, alterations in autonomic nervous system function, a high frequency of subjective complaints, intolerance to alcohol, and increased cardiac output or bradycardia (Bardodej and Vskocil, 1956; Grandjean et al., 1960; Andersson, 1957; Lilis et al., 1969; Konietzko et al., 1978). These effects appeared to increase in frequency with the length of employment and the concentration of exposure. These reports tended to be anecdotal in nature and did not adequately define exposure levels or potential exposure to other chemicals.

Although quantitative data for humans are sparse, some dose-response data for experimental animals are available. In several studies, subchronic exposure of rats to 100 to 400 ppm for several hours a day produced slight behavioral changes (Grandjean, 1960; Goldberg et al., 1964; Silverman and Williams, 1975). Selective destruction of cerebellar Purkinje cells was reported to occur in dogs exposed chronically for up to 8 hours a day to 300 to 500 ppm TCI (Baker, 1958).

The effects of continuous exposure to TCI have been studied only in experimental animals exposed for relatively short periods of time (about

14 weeks). Prendergast et al. (1967) exposed 15 rats (Long-Evans or Sprague-Dawley), 15 Hartley guinea pigs, 3 New Zealand albino rabbits, 3 squirrel monkeys, and 2 beagle dogs to 189 mg/m^3 (35 ppm) TCI of the "highest reagent grade available." The control groups were considerably larger than the treated groups (e.g., 304 control rats; 48 rabbits). Exposure was continuous and lasted 90 days. There were no treatment-related effects on survival, hemotologic values, or gross or histological appearance of the internal organs in any of the species tested. No overt signs of toxicity were observed. A depression of body weight gain was noted in the 3 rabbits tested. Body weight gains of rats and guinea pigs were very slightly depressed. Because body weight gain was reported as a mean percent change from starting weight and no information was given on food or water consumption, the biologic and statistical signficance of the body weight data cannot be assessed.

Two chronic studies with experimental animals were available; both involved intermittent exposure and were concerned primarily with potential carcinogenicity. The first study, conducted for the Manufacturing Chemists Association, had serious shortcomings including marked fluctuations in exposure concentration, lack of dose-monitoring data for much of the study, and inaccurate recording of the observed pathology (Bell et al., 1978). The second study, conducted by Henschler et al. (1980), included very little toxicological data. Rats (Han:Wist), mice (Han: NMRI), and hamsters (Syrian) were exposed to 100 or 500 ppm epoxide-free TCI for 18 months, followed by observation through 30 to 36 months. This exposure regimen had no effect on body weights of any of the species and no effect on survival of the rats and hamsters. Survival of the mice was statistically significantly decreased at both exposure levels in both sexes in a dose-related manner. Hence, intermittent chronic exposure to 100 ppm represents a frank effect level (FEL) in the most sensitive species studied. The only neoplastic effect noted was a dose-related increase in lymphomas in female mice. No information on non-neoplastic pathologic changes was included in the report.

8.2.3 Estimated Threshold Effect Levels

As discussed above, some (but not all) short-term studies with humans and animals indicate that the threshold region for CNS effects may be as low as 100 to 200 ppm. No chronic animal studies that define no-effect or low-effect levels of inhalation exposure were identified. Two subchronic animal studies are available (Kimmerle and Eben, 1973; Prendergast et al., 1967). Although neither one is totally adequate, both are of fairly good quality.

The study by Kimmerle and Eben (1973) was used to determine an inhalation LOEL of 56 ppm (300 mg/m^3), 8 hours/day, 5 days/week, obtained with male Wistar II (SPF) rats (20 treated and 20 controls) treated for 14 weeks. This level of exposure produced increased liver weights (both absolute and relative to body weight) but did not affect weights of other organs, clinical chemistry values related to hepatic and renal function, hematologic values, gross appearance of internal organs, or behavior of the animals. Histopathological examinations were not performed.

Alternatively, a 35 ppm (189 mg/m^3) low or no effect level could be based on the subchronic animal study performed by Prendergast et al. (1967) and summarized in Section 8.2.2. In this study, continuous exposure to 35 ppm TCI for 90 days did not produce any overt signs of toxicity and did not affect survival, hematologic values, or macro or microscopic appearance of the internal organs of rats, guinea pigs, rabbits, dogs, or monkeys. Other advantages of the Prendergast et al. (1967) study are that five species were tested and histopathological examinations of several organs (including liver, kidney, and heart) were done. A potential disadvantage is the uncertainty over the biological and statistical significance of the decreased body weight gain noted in one species.

The inhalation toxicity should be reassessed when the inhalation bioassay of epoxide-free ICI, currently in progress, is completed by Maltoni and associates. This bioassay may provide some chronic toxicity data.

8.3. ORAL EXPOSURE

8.3.1. Effects of Oral Exposure

Virtually no dose-response data for oral exposure of humans to ICI appeared to be available (Chapter 5). Cases of poisoning from ingestion of ICI involved adverse effects on the heart or liver (Defalque, 1961; Secchi et al., 1968). Liver damage was attributed to contamination of the ICI with 1,2-dichloropropane and 1,2-dichloroethane because no such damage occurred when pure ICI was ingested (Secchi et al., 1968).

Toxic dose data in Table 5-1 (taken from the NIOSH Registry of Toxic Effects of Chemical Substances) give some indication of the amount of ICI that might be lethal to humans when ingested. The oral LD_{Lo} for humans of 7000 mg/kg body weight (Table 5-1) is probably at best only a rough estimate of the lower limit of the fatal dose range. Lethal dose data from animals can sometimes be used to calculate an approximate lethal dose for humans using the cubed root of the body weight ratios for interspecies conversions (U.S. EPA, 1980a; Eriereich et al., 1966; Rall, 1969). As generally applied, this extrapolation assumes that larger species are more sensitive than smaller species. Oral LD_{Lo} values for cats, rabbits, and dogs were reported to be 5864, 7330, and 5860 mg/kg body weight, respectively (Table 5-1). These data do not indicate an increase in sensitivity with increasing body weight. Hence, little confidence could be placed in a human lethal dose calculated by this method. Simply for the purposes of comparison with the reported LD_{Lo} for humans, however, estimated human lethal doses were extrapolated from the experimental animal LD_{Lo} values; the range of estimated value was 1790 to 3063 mg/kg body (e.g., 5860 mg/kg x $(2 \text{ kg}/70 \text{ kg})^{1/3}$ = 1790 mg/kg).

Investigations of the effects of prolonged oral administration of TCI to experimental animals include one subchronic toxicity study (Tucker et al., 1980; Sanders et al., 1980) and three carcinogenicity bioassays (National Cancer Institute (NCI), 1976; Van Duuren et al., 1979; Maltoni, 1979).

The subchronic study (Tucker et al., 1980; Sanders et al., 1980), as yet unpublished, is described in the U.S. EPA Carcinogen Assessment Group's appendix (Dec., 1980) to this document. In this study, TCI of 99.9 percent purity was given to male and female CD-1 mice in their drinking water for 4 or 6 months (15/sex/dose/time period). Time-weighted average daily doses of TCI were 19, 220, 395, or 660 mg/kg body weight for the males and 18, 200, 450, or 800 mg/kg body weight for the females. Observations included water consumption, growth, gross pathologic changes, liver and kidney weights, hematology, urinalysis, clinical chemistry, hepatic microsomal enzyme activities, and humoral- and cell-mediated immunologic responses. No toxic effects were found in males and females receiving the lowest dosages, except for slight reductions in indices of immune response in females. This level, 18 mg/kg body weight/day, corresponds to a LOEL. Pathologic abnormalities found in the 3 higher dosage groups involved changes in the macroscopic appearance of the livers, gas pockets and blood in the intestines, and hyperemia of the lungs. Water consumption was reduced in males at the two highest dosage levels. Body weight was unaffected in both sexes at all treatment levels except for a slight but significant reduction in body weights of the highest dose males. Liver weights were increased in some of the higher dosage groups and some hematologic and clinical chemistry values were affected but there was no clear dose response. The effects on immune response were more apparent in females than in males.

The carcinogenicity bioassays contain little useful data on toxicity. In the NCI study, technical grade TCI (containing epichlorohydrin and epoxybutane) was administered by gavage once daily 5 days a week for 78 weeks to Osborne-Mendel rats and B6C3F1 mice of both sexes. The time-weighted average daily doses for the rats were 549 or 1097 mg/kg body weight for the days on which treatment was given. During the last 48 weeks of treatment rats received treatment for only 4 weeks out of every 5 weeks. Body weight gain was depressed and survival was significantly decreased in both high-dose and low-dose groups as compared to controls. No significant toxic hepatic changes were observed. Both groups of treated rats had slight to moderate degenerative and regenerative changes in the renal tubules, primarily affecting the proximal tubular epithelium. On the basis of these results, even the low-dose level of treatment (549 mg/kg body weight/ treatment day) represents a frank-effect level (FEL). A weakness of this study is that survival of the control rats was poor, probably due to intercurrent respiratory disease, which appeared to affect both control and treated groups equally.

The NCI bioassay with mice provides little toxicity data. Male mice received time-weighted average daily doses of 1169 or 2339 mg/kg body weight; female mice received time-weighted average daily doses of 869 or 1739 mg/kg body weight. The mice appeared to be less sensitive to the toxicity of TCI than were the rats as reflected by body weight gains and survival, but no analysis of non-neoplastic pathological changes was given for the mice and the mice apparently were not afflicted with intercurrent disease. The incidence of hepatocellular carcinoma was high in the treated mice, particularly in the males.

Gavage studies by Maltoni (1979) and Van Duuren et al. (1979) using rodents and purified epoxide-free TCI, give virtually no information on toxicity, aside from a statement by Van Duuren et al. (1979) that "The survival of all animals was excellent, except in the positive control groups." (The Van Duuren et al. (1979) study involved the testing of a series of halogenated hydrocarbons, each at a single dosage level.) The NCI is conducting lifetime gavage studies of epoxide-free ICI in B6C3E1 mice and several strains of rats. These studies may provide more information on the chronic oral toxicity of TCI.

8.3.2. Estimated Threshold Level

The Van Duuren et al. (1979) study is one available long-term oral study from which an apparent NOEL or LOEL can be determined. The NCI (1976) gavage study used dosages that were FELs in the more sensitive of the two species tested. Unfortunately, the Van Duuren et al. (1979) study contained virtually no toxicity data, used only one dose level, and used a dosing regimen (gavage, 1 day a week).

The as yet unpublished study reported by Tucker et al. (1980) and Sanders et al. (1980), and summarized in the CAG appendix to this document, would appear to provide a more appropriate basis for determing a low or no effects level. In this study, male and female CD-1 mice were given trichloroethylene of greater than 99.9 percent purity in their drinking water for 4 and 6 months. Consumption of time-weighted average doses of 18 and 19 mg/kg body weight/day by females and males, respectively, produced no potentially adverse changes except for slight decreases in some indices of immune response in females and, hence, represents a LOEL. Higher dosages (200 to 800 mg/kg/day) produced some toxic effects but did not affect survival.

Oral toxicity should be reevaluated when the NCI gavage studies currently in progress are completed.

8.4. DERMAL EXPOSURE

Toxicity from dermal exposure is probably not important in comparison with other routes. Pharmacokinetic studies with human subjects indicate that absorption of liquid TCI through the skin is very slow (Stewart and Dodd, 1964) and absorption of TCI vapor through the skin is usually negligible. Irritation, burns, and rashes have occurred as a result of direct skin contact with the solvent (Section 5.5).

8.5. REFERENCES

Andersson, A. Gesund heitliche Gefahren in der Industrie bei Exposition fur Trichlorathylen. Health dangers in industry from exposure to trichloroethylene. Acta Med. Scand. Suppl. 157(323):1-220, 1957.

Baerg, R.D., and D.V. Kimberg. Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers." Ann. Inter. Med. 73:713-720, 1970.

Bardodej, Z., and J. Vyskocil. The problem of trichloroethylene in occupational medicine: Trichloroethylene metabolism and its effects on the nervous system as a means of hygienic control. AMA Arch. Ind. Health. 13:581-592, 1956.

Bell, Z.G., K.J. Olson, and T.J. Benya. Final report of audit findings of the Manufacturing Chemists Association (MCA): Administered trichloroethylene (TCE) chronic inhalation study at Industrial Bio-test Laboratories, Inc., Decatur, Illinois. Unpublished. 1978.

Brittain, G.J.C. Trichloroethylene (trilene) as anesthetic in neurosurgery. Anesth. and Analg. 27:145-151, 1948.

Clearfield, H. Hepatorenal toxicity from sniffing spot remover (trichloroethylene). Dig. Dis. 15:851-856, 1970.

Defalque, R.J. Pharmacology and toxicology of trichloroethylene: A critical review of the world literature. Clin. Pharmacol. Therap. 2:665-688, 1961.

Dripps, R.D. et al. Introduction to Anesthesia, the Principles of Safe Practice. 5th ed. W.B. Saunders Co., Philadelphia, Pennsylvania, p. 121, 1977. Cited in U.S. EPA, 1980b.

Freireich, E.J., E.A. Gehan, D.P. Rall, L.H. Schmmidt, and H.E. Skipper. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother. Rep. 50:219, 1966.

Gehring, P.J. Hepatotoxic potency of various chlorinated hydrocarbon vapors relative to their narcotic and lethal potencies in mice. Toxicol. Appl. Pharmacol. 13:287-298, 1968.

Goldberg, M.E., H.E. Johnson, U.C. Pozzani, and H.F. Smyth, Jr. Effect of repeated inhalation of vapors of industrial solvents on animal behavior. I. Evaluation of nine solvent vapors and pole-climb performance in rats. Am. Ind. Hyg. Assoc. J. 25:369-375, 1964.

Grandjean, E. The effects of short exposures to trichloroethylene on swimming performance and motor activity of rats. Am. Ind. Hyg. Assoc. J. 24:376-379, 1963.

Grandjean, E. Trichloroethylene effects on animal behavior. Arch. Environ. Health. 1:106-108, 1960.

Henschler, D., W. Romen, H.M. Elasser, D. Reichert, E. Eder, and Z. Radwan. Carcinogenicity study of trichloroethylene by long-term inhalation in the animal species. Arch. Toxicol. 43:237-248, 1980.

Kim, N.K. Air pollution evaluations using risk assessment methodology. Air Pollut. Contr. Assoc. 31(2):120-122, 1981.

Kimmerle, G., and A. Eben. Metabolism, excretion and toxicology of trichloroethylene after inhalation. I. Experimental exposure on rats. Arch. Toxikol. 30:115-126, 1973a.

Kleinfeld, M., and I.R. Tabershaw. Trichloroethylene toxicity: Report of five fatal cases. AMA Arch. Ind. Hyg. Occup. Med. 10:134-141, 1954.

Konietzko, H., W. Haberlandt, H. Heilbronner, G. Reill, and H. Weichardt. Chromosome studies on trichloroethylene workers. Arch. Toxicol. 40:201-206, 1978.

Kylin, B., K. Axell, H.E. Samuel, and A. Lindborg. Effect of inhaled trichloroethylene on the CNS as measured by optokinetic nystagmus. Arch. Environ. Health. 15:48-52, 1967.

Leonardos, G., D. Kendall, and N. Barnard. Odor threshold determinations of 53 odorant chemicals. J. Air Pollut. Contr. Assoc. 19:91-95, 1969.

Lilis, R., D. Stanescu, N. Muica, and A. Roventa. Chronic effects of trichloroethylene exposure. Med. Lav. 60:595-601, 1969.

NAS (National Academy of Sciences). Drinking Water and Health. Safe Drinking Water Committee, Advisory Center on Toxicology, Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington, DC, p. 939. Available from: Printing and Publishing Office, National Academy of Sciences, 2101 Constitution Ave., Washington, DC 20418, 1977.

NCI (National Cancer Institute). Carcinogenesis bioassay of trichloroethylene. National Cancer Institute Carcinogenesis Technical Report Series No. 2, HEW Publication No. NIH 76-802, February 1976. 197 p.

Nomiyama, K., and H. Nomiyama. Trichloroethylene metabolism in man and animals, with special reference to host and agent factors modifying the trichloroethylene metabolism. Ind. Environ. Xenobiot. 2:173-176, 1977.

Prendergast, J.A., R.A. Jones, L.J. Jenkins, Jr., and J. Siegel. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl. Pharmacol. 10:270-289, 1967.

Rall, D.P. Difficulties in extrapolating the results of toxicity studies in laboratory animals to man. Environ. Res. 2:360-367, 1969.

Salvini, M., S. Binaschi, and M. Riva. Evaluation of the psychophysiological functions in humans exposed to trichloroethylene. Br. J. Ind. Med. 28:293-295, 1971.

Sanders, V.M., A.N. Tucker, P.H. Hallett, B. Kaufman, R.A. Carchman, and A.E. Munson. Immunotoxicology of trichloroethylene in the mouse. II Effects on humoral and cell-mediated immunity. Unpublished. 1980.

Secchi, G.C., G. Chiappino, A. Lotto, and N. Zurlo. Actual chemical composition of commerical trilene and their hepatotoxic effects: Clinical enzymologic study. Med. Lav. 59:486-497, 1968.

Silverman, A.P., and H. Williams. Behaviour of rats exposed to trichloroethylene vapours. Br. J. Ind. Med. 32:308-315, 1975.

Stewart, R.D., H.C. Dodd, H.H. Gay, and D.S. Erley. Experimental human exposure to trichloroethylene. Arch. Environ. Health. 20:64-71, 1970.

Stewart, R.D., and H.C. Dodd. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin. Am. Ind. Hyg. Assoc. J. 25:439-446, 1964.

Stopps, G.J., and M. McLaughlin. Psychophysiological testing of human subjects exposed to solvent vapors. Am. Ind. Hyg. Assoc. J. 28:43-50, 1967.

Stuber, K. Gesundheitsschadigen bei der Gewerblichen Verwendung des Irichloroalthylens und die Monglichkelien ihrer Verhutung. Arch. Gewerbepath. Bewerbehyg. 2:398-456, 1931.

Su, G., and K.A. Wurzel. A regulatory framework for setting air emission limits for noncriteria pollutants. J. Air Pollut. Contr. Assoc. 31(2):160-162, 1981.

Tucker, A.N.M., V.M. Sanders, D.W. Barnes, T.J. Bradshaw, and A.E. Munson. Immunotoxicology of trichloroethylene in the mouse. I. General toxicology. Unpublished. 1980.

U.S. EPA (U.S. Environmental Protection Agency). Guidelines and Methodology for the Preparation of Health Effect Assessment Chapters of the Ambient Water Quality Criteria Documents. U.S. EPA, Environmental Criteria and Assessment Office; Office of Health and Environmental Assessment; Office of Research and Development, Cincinnati, OH, November 28, 1980a.

U.S. EPA (U.S. Environmental Protecton Agency). Ambient Water Quality Criteria for Halomethanes. U.S. EPA. Available from: National Iechnical Information Services, Springfield, VA (NTIS PB81-117624), 1980b.

Utesch, R.C., F.W. Weir, and J.V. Bruckner. Development of an animal model of solvent abuse for use in evaluation of extreme trichloroethylene inhalation. Toxicol. 19:169-182, 1981.

Van Duuren, B.L., B.M. Goldschmidt, G. Lowengart, A.C. Smith, S. Melchionne, I. Seldman, and D. Roth. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. J. Natl. Cancer Inst. 63:1433-1439, 1979.

Vernon, R.J., and R.K. Ferguson. Effects of trichloroethylene on visual-motor performance. Arch. Environ. Health. 18:894-900, 1969.

World Health Organization. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, No. 4, Geneva, April 4-12, 1972.

9. COLLATED BIBLIOGRAPHY

Abrahamsom, S., and R. Valencia. Evaluation of substances of interest for genetic damage using Drosophila melanogaster. Final sex-linked recessive lethal test report to FDA on 13 compounds. FDA Contract No. 233-77-2119, 1980.

Adams, E. M., H. C. Spencer, V. K. Rowe, D. D. McCollister, and D. D. Irish. Vapor toxicity of trichloroethylene determined by experiments on laboratory animals. Arch. Ind. Hyg. Occup. Med. 4:469-481, 1951.

Albahary, C., C. Guyotjeannin, A. Flaisler, and P. Thiaucourt. Transaminases et exposition professionnelle au trichloroethylene. Arch. Mal. Prof. Med. Trav. Secur. Soc. 20:421-426, 1959.

Alexander, H. C., W. M. McCarty, and E. A. Bartlett. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bull. Environ. Contam. Toxicol. 20:344-352, 1978.

Allemand, H., D. Pessayre, V. Descatoire, C. Degott, G. Feldman, and J-P. Benhamou. Metabolic activation of trichloroethylene into a chemically reactive metabolite toxic to the liver. J. Pharmacol. Exptl. Therap. 204:714-723, 1978.

Altshuller, A. P. Lifetimes of organic molecules in the troposphere and lower stratosphere. Environ. Sci. Technol., 1980 (in press).

Andersson, A. Gesund heitliche Gefahren in der Industrie bei Exposition fur Trichlorathylen. Health dangers in industry from exposure to trichloroethylene. Acta Med. Scand. Suppl. 157(323):1-220, 1957.

Astrand, I., and P. Ovrum. Exposure to trichloroethylene. I. Uptake and distribution in man. Scand. J. Work Environ. & Health 4:199-211, 1976.

Atkinson, R. S. Trichloroethylene anaesthesia. Anesthesiology 21:67-77, 1960.

Aviado, D. Pharmacologic Principles of Medical Practice. Krantz and Carr, eds. 8th edition, Williams and Wilkens Company, 1972, pp. 251, 1241.

Aviado, D., S. Zakhari, J. Simaan, and A. Ulsamer. Methyl Chloroform and TCE in the Environment. CRC Press, Cleveland, OH, 1976.

Axelson, O., K. Anderson, C. Hogstedt, B. Holmberg, G. Molina, and A. de Verdier. A cohort study on trichloroethylene exposure and cancer mortality. J. Occup. Med. 20:194-196, 1978.

Ayre, P. Acute yellow fever after Trilene anesthesia. Correspondence, Brit M.J. 2:784, 1945.

Baden, J. M., M. Kelley, R. I. Mayze, and V. F. Simmon. Mutagenicity of inhalation anesthetics: Trichloroethylene, divinyl ether, nitrous oxide and cyclopropane. Br. J. Anaesth. 51:417-421, 1979.

Baerg, R. D., and D. V. Kimberg. Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers." Ann. Inter. Med. 73:713-720, 1970.

Baker, A. B. The nervous system in trichloroethylene: an experimental study. Neuropathol. Exp. Neurol. 17:649-655, 1958.

Banerjee, S., and B. L. Van Duuren. Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA *in vitro*. Cancer Res. 38:776-780, 1978.

Bardodej, Z., and J. Vyskocil. The problem of trichloroethylene in occupational medicine: trichloroethylene metabolism and its effects on the nervous system as a means of hygienic control. AMA Arch. Ind. Health 13:581-592, 1956.

Barnes, C. G., and J. Ives. Electrocardiographic changes during trilene anesthesia. Proc. Roy. Soc. Med. 37:528-532, 1944.

Barrett, H. M., and J. H. Johnston. The fate of trichloroethylene in the organism. J. Biol. Chem. 127:765-770, 1939.

Barrett, H. M., J. C. Cunningham, and J. H. Johnston. Study of fate in organism of some chlorinated hydrocarbons. J. Indust. Hyg. Toxicol. 21:479-490, 1939.

Bartonicek, V. Metabolism and excretion of trichloroethylene after inhalation by human subjects. Brit. J. Ind. Med. 19:134-141, 1962.

Bartonicek, V., and J. Teisinger. Effect of tetraethyl thuiram disulphide (disulfiram) on metabolism of trichloroethylene in man. Brit. J. Ind. Med. 19:216-221, 1962.

Bartonicek, V. J., and A. Brun. Subacute and chronic trichloroethylene poisoning: a neuropathological study in rabbits. Acta Pharmacol. Toxicol. 28:359-369, 1970.

Bartsch, H., C. Malaveille, A. Barkin, and G. Planche. Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes and dichlorobutanes produced by rodent or human liver tissues. Arch. Toxicol. 41:249-278, 1979.

Batelle Columbus Laboratories. Environmental Monitoring Near Industrial Sites: Trichloroethylene. Prepared for the U.S. Environmental Protection Agency, PB 273203, August 1977.

Battig, K., and E. Grandjean. Chronic effects of trichloroethylene on rat behavior. Arch. Environ. Hlth. 7:694-699, 1963.

Bauer, M., and S. F. Rabens. Cutaneous manifestations of trichloroethylene toxicity. Arch. Dermatol. 110:886-890, 1974.

Beckers, N. L. Stabilization of Chlorinated Hydrocarbons. Patent No. 3,682,830, U.S. Patent Office, Washington, DC, August 8, 1972.

Beliles, R. P., D. J. Brusick, and E. J. Mecler. Teratogenic-Mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene and carbon disulfide. U.S. Department of Health, Education and Welfare (Contract No. 210-77-0047), 1980.

Bell, A. Death from trichloroethylene in a dry-cleaning establishment. N.Z. Med. J. 50:119-126, 1951.

Bell, Z. Written communication with contractor reports of: (a) Dominant Lethal Study with Trichloroethylene in Albino Rats Exposed via Inhalation, February 9, 1977, and (b) Teratogenic Study via Inhalation with Trichlor 132, Trichloroethylene in Albino Rats, March 8, 1977.

Bell, Z. G., K. J. Olson, and I. J. Benya. Final report of audit findings of the Manufacturing Chemists Association (MCA): Administered trichloroethylene (TCE) chronic inhalation study at Industrial Bio-test Laboratories, Inc., Decatur, Illinois. Unpublished. 1978.

Bellar, T. A., J. J. Lichtenberg, and R. C. Kroner. The occurrence of organohalides in chlorinated drinking waters. J. Amer. Water Works Assoc. 66:703-706, 1974.

Bellar, T. A., W. L. Budde, and J. W. Eichelberger. The Identification and Measurement of Volatile Organic Compounds in Aqueous Environmental Samples. Chapter 4. In: Monitoring Toxic Substances. D. Schvetzle, ed., ACS Symposium Series 94, American Chemical Society, 1979.

Berleb, M., Eklampsie und Trichloran-Analgesie, München. med. Wehnschr. 13:1225, 1953.

Bernstine, J. B., A. E. Meyer, and H. B. Hayman. Maternal and foetal blood estimation following the administration of chloral hydrate during labour. J. Obst. Gyn. 61:683-685, 1954.

Bernstine, J. B., A. E. Meyer, and R. L. Bernstine. Maternal blood and breast milk estimation following the administration of chloral hydrate during the puerperium. J. Obst. Gyn. 63:228-231, 1956.

Bernstine, J. B., and A. E. Meyer. Passage of metabolites of chloral hydrate into amniotic fluid. Proc. Soc. Exp. Biol. Med. 84:456-457, 1953.

Bernstine, M. L. Cardiac arrest occurring under trichloroethylene analgesia. AMA Arch. Surg. 68:262-266, 1954.

Blair, A. H., and E. H. Bodley. Human liver aldehyde dehydrogenase. Partial purification and properties. Can. J. Biochem. 47:265-271, 1969.

Blair, A., P. Decoufle, and D. Grauman. Causes of death among laundry and dry cleaning workers. Am. J. Publ. Hlth. 69:508-511, 1979.

Bolt, H. M., A. Buchter, L. Wolowski, D. L. Gil, and W. Bolt. Incubation of ^{14}C-trichloroethylene vapor with rat liver microsomes. Uptake of radioactivity and covalent protein binding of metabolites Int. Arch. Occup. Environ. Health 39:103-111, 1977.

Bolt, H. M., and J. G. Eilser. Irreversible binding of chlorinated ethylenes to macromolecules. Environ. Hlth. Persp. 21:107-112, 1977.

Bonse, G., and D. Henschler. Chemical reactivity, biotransformation, and toxicity of polychlorinated aliphatic compounds. CRC Crit. Rev. Toxicol. 4:395-409, 1976.

Bonse, G., I. Urban, D. Reichert, and D. Henschler. Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem. Pharmacol. 24:1829-1834, 1975.

Borthwick, P. W. Results of toxicity tests with fishes and macroinvertebrates. Data sheets available from U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL, 1977.

Boulton, T. B., and R. B. Sweet. The place of trichloroethylene in modern anesthesia. J. Mich. State Med. Soc. 59:270-273, 1960.

Braddock, J. 1976. Cited in: Waters, E.M., H.B. Gerstner, and J.E. Huff. Trichloroethylene. I. An overview. J. Toxicol. Environ. Hlth. 2:671-707, 1977.

Briemer, D., H. C. Ketclaars, and I. M. von Rossum. Gas chromatographic determination of chloral hydrate, trichloroethanol and trichloroacetic acid in blood and in urine employing head-space analysis. J. Chromatogr. 88:55-63, 1974.

British Department of the Environment. Chlorofluorocarbons and Their Effect on Stratospheric Ozone (Second Report). Pollution Paper No. 15, Her Majesty's Stationery Office, 1979.

Brittain, G. J. C. Trichloroethylene (trilene) as anesthetic in neurosurgery. Anesth. & Analg. 27:145-151, 1948.

Bronzetti, G., E. Zeiger, and D. Erezza. Genetic activity of trichloroethylene in yeast. J. Environ. Pathol. and Toxicol. 1:411-418, 1978.

Brown, N. A., E. H. Goulding, and S. Fabro. Ethanol embryo toxicity: direct effects on mammalian embryos in vitro. Science 206:573-575, 1979.

Bucon, H.W., J. E. Macko, and H. J. Taback. Volatile Organic Compound (VOC) Species Data Manual. EPA-450/3-78-119. Report prepared for the U.S. Environmental Protection Agency, December 1978.

Buelke-Sam, J., and C. A. Kimmel. Development and standardization of screening methods for behavioral teratology. Teratology 20:17-30, 1979.

Butler, T. C. Metabolic fate of chloral hydrate. J. Pharmacol Exptl. Therap. 92:49-58, 1948.

Butler, T. C. Metabolic transformation of trichloroethylene. J Pharmacol. Exptl. Therap. 97:84-92, 1949.

Buttner, H. Aldehyde and alcohol dehydrogenase activity in liver and kidney of the rat. Biochem. 2. 341:300-314, 1965.

Byington, K. H., and K. C. Leibman. Metabolism of trichloroethylene in liver microsomes. II. Identification of the reaction product as chloral hydrate. Mol. Pharmacol. 1:247-254, 1965.

Callen, D. F., C. R. Wolf, and R. M. Philpot. Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccaromyces cerevisiae*. Mutat. Res. 77:55-63, 1980.

Campbell, M. J., J. C. Sheppard, and B. E. Au. Measurement of hydroxyl radical concentrations in boundary layer air by monitoring CO oxidations. Geophy. Res. Lett. 6:175-178, 1979.

Canada, Ministry of Health and Welfare. The testing of chemicals for carcinogenicity, mutagenicity, and teratogenicity. 1973.

Carcinogen Assessment Group Report on Trichloroethylene External Review Draft. April, 1981.

Carlson, G. P. Enhancement of the hepatotoxicity of trichloroethylene by inducers of drug metabolism. Res. Commun. Chem. Pathol. Pharmacol. 7:637-640, 1974.

Chemical and Engineering News, 19 May 1975, pp. 41-43.

Chemical Marketing Reporter. Chemical Profile for Trichloroethylene, 26 June 1978.

Chernoff, N. Personal communication, and published in, U.S. EPA Office of Pesticides. Notice of determination: Abstract of Carbaryl Decision Document. Federal Register 45:81869, December 12, 1980.

Clarren, S. K., and D. W. Smith. The fetal alcohol syndrome. New Eng. J. Med. 298:1063-1067, 1978.

Clearfield, H. Hepatorenal toxicity from sniffing spot remover (trichloroethylene). Dig. Dis. 15:851-856, 1970.

Cole, W. J., R. G. Mitchell, and R. E. Salamonsen. Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. J. Pharm Pharmcol. 27:167, 1975.

Coleman, W. E., R. D. Lingg, R. G. Melton, and F. C. Kopfler. The Occurrence of Volatile Organics in Five Drinking Water Supplies Using Gas Chromatography/Mass Spectrometry. Chapter 21. In: Identification and Analysis of Organic Pollutants in Water. L.H. Keith, ed., Ann Arbor Science, 1976.

Coleridge, H. M., J. C. G. Coleridge, J. C. Luck, and J. Norman. The effect of four volatile anaesthetic agents on the impulse activity of two types of pulmonary receptor. Br. J. Anaesth. 40:484-492, 1968.

Committee on Methods for Toxicity Tests with Aquatic Organisms: Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Series, EPA-600/3-75-009, 1975.

Conkle, J. P., B. J. Camp, and B. E. Welch. Trace composition of human respiratory gas. Arch. Environ. Hlth. 30:290-295, 1975.

Cooper, J. R., and P. J. Friedman. The enzymic oxidation of chloral hydrate to trichloroacetic acid. Biochem. Pharmacol. 1:76-82, 1958.

Corbett, T., G. Hamilton, M. Yoon, and J. Endres. Occupational exposure of operating room personnel to trichloroethylene. Can. Anesth. Soc. J. 20:657-678, 1973.

Corbett, T. H. Anesthetics as a cause of abortion. Fert. Steril. 23:866-869, 1972.

Corbett, T. H., R. G. Cornell, J. L. Endres, and K. Leiding. Birth defects among children of nurse anesthetists. Anesthesiology 41:341-344, 1974.

Cornish, H. H., and J. Adefuin. Ethanol potentiation of halogenated aliphatic solvent toxicity. Am. Ind. Hyg. Ass. J. 27:57-61, 1966.

Cornish, H. H., M. L. Barth, and B. Ling. Influence of aliphatic alcohols on the hepatic response to halogenated olefins. Environ. Health Persp. 21:149-152, 1977.

Correia, Y., G. J. Martens, F. H. van Mensch, and B. P. Whim. The occurrence of trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane in Western Europe in air and water. Atmos. Environ. 11:1113-1116, 1977.

Cote, C. J. Birth defects among infants of nurse anesthetists. Anesthesiology 42:514-515, 1975.

Cotter, L. H. Trichloroethylene poisoning. Arch. Ind. Hyg. Occup. Med. 1:319-322, 1960.

Creech, J. L., Jr., and M. N. Johnson. Angiosarcoma of the liver in the manufacture of polyvinyl chloride. J. Occup. Med. 16:150-151, 1974.

Cronn, D. R., and D. E. Harsch. Determination of atmospheric halocarbon concentrations by gas chromatography-mass spectrometry. Anal. Lett. 12(B14):1489-1496, 1979.

Cronn, D. R., and E. Robinson. Determination of Trace Gases in Learjet and V-2 Whole-air Samples Collected During the Intertropical Convergence Zone Study. Report submitted to NASA, Washington State University, August 1978.

Cronn, D. R., R. A. Rasmussen, and E. Robinson. Report for Phase I. Report prepared for U.S.E.P.A., Contract No. R0804033-01, Washington State University, 23 August 1976.

Cronn, D. R., R. A. Rasmussen, and E. Robinson. Measurement of Tropospheric Halocarbons by Gas Chromatography/Mass Spectrometry. Report for Phase II of EPA Grant No. R0804033-02, October 1977b.

Cronn, D. R., R. A. Rasmussen, E. Robinson, and D. E. Harsch. Halogenated compound identification and measurement in the troposphere and low stratosphere. J. Geophy. Res. 82:5935-5943, 1977a.

Crutzen, P., and J. Eishman. Average concentrations of OH in the troposphere and the budgets of CH_4, CO, H_2 and Ch_3CCl_3. Geophy. Res. Lett. 4:321-324, 1977.

Crutzen, P. A., I. S. A. Isaksen, and J. R. McAfee. The impact of the chlorocarbon industry on the ozone layer. J. Geophy. Res. 83:345-362, 1978.

Cundy, J. M. Trichloroethylene in anesthetic practice. Anesthesia 31:950 (Letter), 1976.

Dahlberg, J. A. The non-sensitized photooxidation of trichloroethylene in air Acta Chem. Scand. 23:3081-3090, 1969.

Dalbey, W., and E. Bingham. Metabolism of Trichloroethylene by the Isolated Perfused Lung. Toxicol. Appl. Phamacol. 43:267-277, 1978.

Daniel, J. W. The metabolism of ^{36}Cl-labelled trichloroethylene and tetrachloroethylene in the rat. Biochem. Pharmacol. 12:795-802, 1963.

Defalque, R. J. Pharmacology and toxicology of trichloroethylene: A critical review of the world literature. Clin. Pharmacol. Therap. 2:665-688, 1961.

Demopoulos, H. B., B. Wagner, and J. Cimino. An academic review of the hazards posed by trichloroethylene. Unpublished document presented at EPA Science Advisory Board meeting on Air Carcinogens, Washington, DC, Sept. 3-4, 1980.

Derwent, R. G., and A. E. J. Eggleton. Halocarbon lifetimes and concentration distributions calculated using a two-dimensional tropospheric model. Atmos. Environ. 12:1261-1269, 1978.

Dhuner, K. G. Cardiac irregularities due to trichloroethylene given during labor. Acta Obst. Ct Gynee. Scandinav. 31:478-482, 1951.

Dhuner, K. G. Cardiac irregularities in trichloroethylene poisoning. Acta. anaesth. Scandinav. 1:121-135, 1957.

Dietz, E. A., and K. F. Singley. Determination of chlorinated hydrocarbons in water by headspace gas chromatography. Anal. Chem. 51:1809-1814, 1979.

DiGiovanni, A. J. Reversal of chloral hydrate. Associated cardiac arrhythmia by a bita-adrenegic blocking agent. Anesthesiology 31:93, 1969.

Dilling, W. L. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11:405-409, 1977.

Dilling, W. L., C. J. Bredeweg, and N. B. Tefertiller. Simulated atmospheric photodecomposition rates of methylene chloride, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other compounds. Environ. Sci. Technol. 10:351-356, 1976.

Dilling, W. L., N. B. Tefertiller, and G. J. Kallos. Evaporation rates and reactivities of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other chlorinted compounds in dilute aqueous solutions. Environ. Sci. Technol. 9:833-838, 1975.

Dobkin A., and P. Byles. Trichloroethylene anesthesia. Clin. Anesth. 1:44-65, 1963.

Dodds, G. H. Necrosis of liver and bilateral massive suprarenal hemorrhage in puerperium, Brit. M. J. 1:769-770, 1945.

Dorfmueller, M. A., S. P. Henne, R. G. York, R. L. Bornschein, and J. M. Manson. Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene. Toxicology, 14:153-166, 1979.

Dowty, B. J., D. R. Carlisle, and J. L. Laseter. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environ. Sci. Technol. 9:762-765, 1975.

Dripps, R. D. et al. Introduction to Anesthesia, the Principles of Safe Practice, 5th ed. W. B. Saunders Co., Philadelphia, Pennsylvania, p. 121, 1977. Cited in U. S. EPA, 1980b.

Eger, E. I., and C. P. Larson. Anesthetic solubility in blood and tissues; values and significance. Brit. J. Anaesth. 36:140-149, 1964.

Elam, J. Correspondence. Brit. M. J. 1:546-547, 1949.

Elovaara, E., K. Hemminki, and H. Vainio. Effects of methylene chloride, trichloroethane, trichloroethylene, tetrachloroethylene and toluene on the development of chick embryos. Toxicology 12:111-119, 1979.

Ertle, T., D. Henschler, G. Muller, and M. Spassowski. Metabolism of trichloroethylene in man. I. The Significance of trichloroethanol in long-term exposure conditions. Arch. Toxikol. 29:171-188, 1972.

Ewing, B. B., E. S. K. Chian, J. C. Cook, C. A. Evans, P. K. Hopke, and E. G. Perkins. Monitoring to Detect Previously Unrecognized p Mutants in Surface Waters. EPA-560/6-77-015. Report prepared by the University of Illinois for the U.S. Environmental Protection Agency, July 1977.

Fahrig, R. The mammalian spot test (Fell fleckentest) with mice. Arch. Toxicol. 38:87-98, 1977.

Farm Chemicals Handbook, 1976. Cited in: Waters, E.M., H.B. Gerstner and J.E. Huff. Trichloroethylene. I. An overview. J. Toxicol. Environ. Hlth. 2:671-707, 1977.

Federal Register 42(131):35314-35316, 8 July 1977.

Ferguson, R. K., and R. J. Vernon. Trichloroethylene in combination with CNS drugs. Effects on visual-motor tests. Arch. Environ. Hlth. 2:462-467, 1973.

Fernandez, J. G., B. E. Humbert, P. O. Droz, and J. R. Caperos. Exposition au trichlorethylene, Bilan de l'absorption, de l'excretion et du metabolisme sur des sujets humains. Arch. Mal. Prof. 36:(7-8)397-407, 1975.

Fernandez, J. G., P. O. Droz, B. E. Humbert, and J. R. Caperos. Trichloroethylene exposure. Simulation of uptake, excretion, and metabolism using a mathematical model. Brit. J. Ind. Med. 34:43-55, 1977.

Fink, R. Toxicity of Anesthetics. Williams and Wilkins Co., Baltimore, MD, p. 270, 1968.

Fishbein, L. Industrial mutagens and potential mutagens. I. Halogenated aliphatic derivatives. Mutat. Res. 32:267-, 1976.

Fishbein, L. Potential halogenated industrial carcinogenic and mutagenic chemicals. I. Halogenated unsaturated hydrocarbons. Sci. Total Environ. 11:111-161, 1979.

Food and Drug Administration Advisory Protocols for Safety Evaluation. Panel and Carcinogenesis report on Cancer Testing in the Safety of Food Additives and Pesticides. Toxicol. Appl. Pharmacol. 20:419-438, 1971.

Forssman, S., and C. E. Holmqvist. The relation between inhaled and exhaled trichloroethylene and trichloroacetic acid excreted in the urine of rats exposed to trichloroethylene. Acta Pharmacol et Toxicol. 9:235-244, 1953.

Friedman, P. J., and J. R. Cooper. The role of alcohol dehydrogenase in the metabolism of chloral hydrate. J. Pharmacol. Exptl. Therap. 129:373-376, 1960.

Freireich, E. J., E. A. Gehan, D. P. Rall, L. H. Schmmidt, and H. E. Skipper. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother. Rep. 50:219, 1966

Fujiwara, K. Sitzungsker u. Abhandl naturforsch. Ges. Postock. 6:33, 1914.

Fuller, B. B. Air pollution assessment of trichloroethylene. MITRE Technical Report No. MTR-7142, 1976.

Gay, B. W., P. L. Hanst, J. J. Bufalini, and R. C. Noonan. Atmospheric oxidation of chlorinated ethylenes. Environ. Sci. Technol. 10:58-67, 1976.

Gehring, P. J. Hepatotoxic potency of various chlorinated hydrocarbon vapors relative to their narcotic and lethal potencies in mice. Toxicol. Appl. Pharmacol. 13:287-298, 1968.

General Industry Standards. OSHA Safety and Health Standards, (29 CFR 1910) United States Department of Labor Occupational Safety and Health Administration OSHA 2206, revised November 7, 1978.

Gessner, P. D., and B. E. Cabana. A study of the interaction of the hypnotic effects and of the toxic effects of chloral hydrate and ethanol. J. Pharmacol. Exp. Therap. 174:247-259, 1970.

Goldberg, M. E., H. E. Johnson, U. C. Pozzani, and H. F. Smyth, Jr. Effect of repeated inhalation of vapors of industrial solvents on animal behavior. I. Evaluation of nine solvent vapors and pole-climb performance in rats. Am. Ind. Hyg. Assoc. J. 25:369-375, 1964.

Goodman, L. S., and A. Gilman. The Pharmacological Basis of Therapeutics. 5th ed. New York: Macmillan, 1975.

Goodman and Gilman's, The Pharmacological Basis of Therapeutics, A. G. Gilman, L. S. Goodman and A. Gilman, eds., 6th ed. New York: MacMillan Pub. Co., 1980. pp. 361-363.

Graedel, T. E., and D. L. Allara. Tropospheric halocarbons: Estimates of atmospheric chemical production. Atmos. Environ. 10:385-388, 1976.

Grandjean, E. The effects of short exposures to trichloroethylene on swimming performances and motor activity of rats. Am. Ind. Hyg. Assoc. J. 24:376-379, 1963.

Grandjean, E. Trichloroethylene effects on animal behavior. Arch. Environ. Hlth. 1:106-108, 1960.

Greim, H., G. Bonse, Z. Radwan, D. Reichert, and D. Henschler. Mutagenicity and chromosomal aberrations as an analytical tool for in vitro detection of mammalian enzyme-mediated formation of reactive metabolites. Arch. Toxicol. 39:159-169, 1977.

Greim, H., G. Bonse, Z. Radwan, D. Reichert, and D. Henschler Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochem Pharmacol 24:2012-2017, 1975.

Grimsrud, E. P., and R. A. Rasmussen. Survey and analysis of halocarbons in the atmosphere by gas chromatography-mass spectrometry. Atmos. Environ. 9:1014-1017, 1975.

Grunett, N. Oxidation of acetaldehyde by rat liver mitochondria in relation to ethanol oxidation and the transport of reducing equivalents across the mitochondrial membrane. Eur. J. Biochem. 35:236-243, 1973.

Gu, Z. W., B. Sele, P. Jalbert, M. Vincent, F. Vincent, C. Marka, D. Chmara, and J. Faure. Induction d'echanges entre les chromatides soeurs (SCE) par le trichloréfthyléne et ses métabolites. Toxicologie Métabolique 3:63-67, 1981.

Guyotjeannin, C., and J. Van Steenkiste. Action of trichloroethylene on proteins and lipoproteins. Study of 18 workers working in polluted atmosphere. Arch. Mal. Prof. Med. Trav. Secur. Soc. 19:489-494, 1958.

Hall, K. D., L. N. Garlington, W. K. Nowill, and C. R. Stephen. The analysis of small concentrations of trichloroethylene vapor by interferometry. Anesth. 14:38-45, 1953.

Hardie, D. W. E. Chlorocarbons and Chlorohydrocarbons: Trichloroethylene. *In*: Kirk-Othmer's Encyclopedia of Chemical Technology. Vol. 5. Second edition, 1964. pp. 183-195.

Harsch, D. Study of Halocarbon Concentrations in Indoor Environments. Report to U.S. Environmental Protection Agency. Contract No. WA-6-99-2922-J. July 7, 1977.

Harsch, D. E., and D. R. Cronn. Low-pressure sample-transfer technique for analysis of stratospheric air samples. J. Chromat. Sci. 16:363-367, 1978.

Heizer, H. Trichloraethylene in der Gebutshilfe, München. Med. Wehnschr. 93:2046-2050, 1951.

Helliwell, P. J., and A. M. Hutton. Trichloroethylene anesthesia. I. Distribution in the fetal and maternal circulation of pregnant sheep and goats. Anaes. 5:4-15, 1950.

Henschler, D. Metabolism and mutagenicity of halogenated olefins - a comparison of structure and activity. Environ. Health Persp. 21:61-64, 1977.

Henschler, D., and G. Bonse. Metabolic activation of chlorinated ethylenes. Dependence of mutagenic effect on electrophilic reactivity of the metabolically formed epoxides. Arch. Toxicol. 39:7-12, 1977.

Henschler, D., E. Eder, T. Neudecker, and M. Metzler. Carcinogenicity of trichloroethylene: Fact or artifact? Arch. Toxicol. 37:233-236, 1977.

Henschler, D., W. Romen, H.M. Elasser, D. Reichert, E. Eder, and Z. Radwan. Carcinogenicity study of trichloroethylene by long-term inhalation in the animal species. Arch. Toxicol. 43:237-248, 1980.

Huff, J. E. New evidence on the old problems of trichloroethylene. Ind. Med. Surg. 40:25-33, 1971.

Humphrey, J. H., and M. McClelland. Cranial-nerve palsies with herpes following general anaesthesia: a report. Br. Med. J. 1:315-318, 1944.

Ikeda, M. Metabolism of trichloroethylene and tetrachloroethylene in human subjects. Environ. Health Persp. 21:239-245, 1977.

Ikeda, M., and T. Imamura. Biological half-life of trichloroethylene and tetrachloroethylene in human subjects. Int. Arch. Arbeitsmed. 31:209-224, 1973.

Ikeda, M., H. Ohtsuji, T. Imamura, and Y. Komoike. Urinary excretion of total trichloro-compounds, trichloroethanol, and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. Brit. J. Ind. Med. 29:328-333, 1972.

Ikeda, T., C. Nagano, and A. Okada. Hepatotoxic effect of trichloroethylene and perchloroethylene in the rat and mouse. Igaku to Seibutsugaku 79:123-129, 1969.

International Agency for Research on Cancer (IARC). Trichloroethylene. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. 20:545-572, 1979.

Ismailov, A. S., and G. V. Ryskal. Mutagenic effect of TCE in the offspring of the fly Drosophila melanogaster. Biol. Nauk. Iss. 1:6-10, 1976.

James, W. R. L. Fatal addiction to trichloroethylene. Brit. J. Ind. Med. 20:47-49, 1963.

Johnson, E. M. Screening for teratogenic hazards, nature of the problems. Ann. Rev. Pharmacol. Toxicol. 21:417-429, 1981.

Jonak, D. Daily and Sunday Herald, (series of articles), 1980.

Jones, G., and G. Scott. U.S. Bureau of Mines--Report of Investigation 3666, Washington, D.C., 1942. Cited in: Kirk-Othmer's Encyclopedia of Chemical Technology. Volume 5. Third Edition, 1979.

Joron, G. E., D. G. Cameron, and G. W. Halpenny. Massive necrosis of the liver due to trichloroethylene. Can. Med. Assoc. J. 73:890-891, 1955.

Jungclaus, G. A., V. Lopez-Avila, and R. A. Hites. Organic compounds in an industrial wastewater. A case study of their environmental impact. Environ. Sci. Technol. 12:88-96, 1978.

Karnofsky, D. A. Drugs as teratogens in animals and man. Ann. Rev. Pharmacol. 5:447-472, 1965a.

Karnofsky, D. A. The chick embryo in drug screening; survey of teratological effects observed in the 4-day chick embryo. In: Teratology, Principles and Techniques, Wilson, J. G. and Warkany, J., eds. Un. Chicago Press, Chicago, Ill., pp: 194-213, 1965b.

Katz, R. M., and D. Jowett. Female laundry and dry cleaning workers in Wisconsin: A mortality analysis. A.J.P.H. 71:305-307, 1981.

Kelley, J. M., and B. R. Brown, Jr. Biotransformation of trichloroethylene. Intern. Anesthesiol. Clin. 12:85-92, 1974.

Kim, N. K. Air pollution evaluations using risk assessment methodology. Air Pollut. Contr. Assoc. 31(2):120-122, 1981.

Kimmerle, G., and A. Eben. Metabolic studies of trichloroethylene. II. Experimental human exposure. Arch. Toxicol. 30:127-138, 1973b.

Kimmerle, G., and A. Eben. Metabolism, excretion and toxicology of trichloroethylene after inhalation. I. Experimental exposure on rats. Arch. Toxikol. 30:115-126, 1973a.

Klaassen, C. D., and G. L. Plaa. Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol. Appl. Pharmacol. 10:119-131, 1967.

Kledecki, Z., and H. Bura. Ostre zatrucia wywolane polknieciem trojehloroethylenu ("Tri"). Pol. Tyg. Lek. 18:748-750, 1963.

Kleinfeld, M., and I.R. Tabershaw. Trichloroethylene toxicity: report of five fatal cases. AMA Arch. Ind. Hyg. Occup. Med. 10:134-141, 1954.

Kline, S. A., and B. L. Van Duuren. Reaction of epoxy-1,1,2-trichloroethane with nucleophiles. J. Heterocycl. Chem. 14:455-458, 1977.

Knoll, J. E., M. A. Smith, and M. Rodney Midget. Evaluation of emission test methods for halogenated hydrocarbons. Vol. I. EPA-600/4-79-025, March 1979.

Konietzko, H., W. Haberlandt, H. Heilbronner, G. Reill, and H. Weichardt. Chromosome studies on trichloroethylene workers. Arch. Toxicol. 40:201-206, 1978.

Kopfler, F. C., R. G. Melton, R. D. Lingg, and W. E. Coleman. In: Identification and Analysis of Organic Pollutants in Water. L.W. Keith, ed., Ann Arbor Science, 1976, pp. 87-104.

Kraemer, R. J., and R. A. Deitrich. Isolation and characterization of human liver aldehyde dehydrogenase. J. Biol. Chem. 243:6402-6408, 1968.

Kucera, J. Exposure to fat solvents: a possible cause of sacral agenesis in man. J. Pediatr. 72:857-859, 1968.

Kummert, R., E. Molnar-Kubica, and W. Giger. Trace determination of tetrachloroethylene in natural water by direct aqueous injection, high-pressure liquid chromatography. Anal. Chem. 50:1637-1639, 1978.

Kunz, E., and R. Isenschmid. The toxic effect of trichloroethylene on the eye. Klin Monatsbl Augenheilkd. 94:577-585, 1935.

Kylin, B., H. Reichard, I. Sumergi, and S. Yllner. Hepatotoxicity of inhaled trichloroethylene, tetrachloroethylene, and chloroform. Single exposure. Acta Pharmacol. Toxicol. 20:16-26, 1963.

Kylin, B., K. Axell, H.E. Samuel, and A. Lindborg. Effect of inhaled trichloroethylene on the CNS as measured by optokinetic nystagmus. Arch. Environ. Hlth. 15:48-52, 1967.

Lachnit, V., and G. Brichta. Trichloroethylene and liver damage. Zbl. Arbeitsmed. 8:56-62, 1958.

Laham, S. Studies on placental transfer: trichloroethylene. Ind. Med. 39:46-49, 1970.

Laib, R. J., G. Stockle, H. M. Bolt, and W. Kung. Vinyl chloride and trichloroethylene: Comparison of alkylating effects of metabolites and induction of preneoplastic enzyme deficiencies in rats livers. J. Can. Res. Clin. Oncol. 94:139-147, 1979.

Laskin, S., A. R. Sellakumar, M. Kuschner, S. LeMendola, N. Nelson, G. M. Rusch, G. V. Katz, N. C. Dulak, and R. E. Albert. Inhalation carcinogenicity of epichlorohydrin in noninbred Sprague-Dawley rats. J. Natl. Cancer Inst. 65:751-755, 1980.

Lehmann, K. B. Experimental studies on the technical influence and essential hygienic gases and steam on the organism. Arch. Hyg. 74:1-60, 1911.

Leibman, K. C., and E. Ortiz. Metabolism of halogenated ethylenes. Environ. Health Persp. 21:91-97, 1977.

Leibman, K. C., and W. J. McAllister, Jr. Metabolism of trichloroethylene in liver microsomes. III. Induction of the enzymic activity and its effect on excretion of metabolites. J. Pharmacol. Exptl. Therap. 157:574-580, 1967.

Leong, B. K. J., B. A. Schwetz, and P. J. Gehring. Embryo- and fetotoxicity of inhaled trichloroethylene, perchloroethylene, methylchloroform and methylene chloride in mice and rats. Toxicol. Appl. Pharmacol. 33(1):136 (Abstract 34), 1975.

Leonardos, G., D. Kendall, and N. Barnard. Odor threshold determinations of 53 odorant chemicals. J. Air Pollut. Contr. Assoc. 19:91-95, 1969.

Liebler, C. S., and L. M. DeCarli. Hepatic microsomes, a new site for ethanol oxidation. J. Clin. Invest. 47:62 (Abst), 1969.

Lilis, R., D. Stanescu, N. Muica, and A. Roventa. Chronic effects of trichloroethylene exposure. Med. Lav. 60:595-601, 1969.

Lillian, D., H. B. Singh, A. Appleby, L. Lobban, R. Arnts, R. Gumpert, R. Hague, J. Toomey, J. Kazazis, M. Antell, D. Hansen, and B. Scott. Atmosphere fates of halogenated compounds. Environ. Sci. Technol. 9:1042-1048, 1975.

Lillian, D., H. B. Singh, and A. Appleby. Gas chromatographic analysis of ambient halogenated compounds. J. Air Pollut. Cont. Assoc. 26:141-143, 1976.

Lin, R. S., and I. I. Kessler. A multifactorial model for pancreatic cancer in man. J.A.M.A. 245:147-152, 1981.

Litt, K. F., and M. I. Cohen. Danger---vapor harmful. Spot-remover sniffing. N. Engl J. Med. 281:543-544, 1969.

Loprieno, N., R. Barale, A. M. Rossi, S. Fumerjo, G. Meriggi, A. Mondino, and S. Silvestri. *In vivo* mutagenicity studies with trichloroethylene and other solvents (preliminary results). Unpublished. 1979.

Lovelock, J. F. Atmospheric halocarbons and stratospheric ozone. Nature 252:292-294, 1974a.

Lovelock, J. F. The electron capture detector: Theory and practice. J. Chromat. 99:3-12, 1974b.

Lowry, L. K., R. Vandervort, and P. L. Polakoff. Biological indicators of occupational exposure to trichloroethylene. J. Occup. Med. 16:98-101, 1974.

Makide, Y., Y. Kanai, and T. Tominaga. Background atmospheric concentrations of halogenated hydrocarbons in Japan. Bull. Chem. Soc. Japan 53:2681-2682, 1980.

Maloof, C. C. Burns of the skin produced by trichloroethylene vapors at room temperature. J. Ind. Hyg. Toxicol. 31:295-296, 1949.

Maltoni, C., and G. Lefemine. Carcinogenicity bioassays of vinyl chloride: current results. Ann. NY Acad. Sci. 246:195-218, 1975.

Maltoni, C. Results of long-term carcinogenicity bioassays of trichloroethylene experiments by oral administration on Sprague-Dawley rats. In press, 1979.

Manson, J. Personal communication, M. S. Christian, May 8, 1981.

Mapleson, W. W. An electric analogue for uptake and exchange of inert gases and other agents. J. Appl. Physiol. 18:197-204, 1963.

Margard, W. Summary report on *in vivo* bioassay of chlorinated hydrocarbon solvents. Unpublished. 1978.

Marshall, E. K., and A. H. Owens. Absorption, excretion and metabolic fate of chloral hydrate. Bull. Johns Hopkins Hosp. 95:1-18, 1954.

Mazza, V., and A. Brancaccio. Characteristics of the formed elements of the blood and bone marrow in experimental trichloroethylene intoxication. Folia. Med. 50:318-324, 1967.

McBirney, R. S. Trichloroethylene and dichloroethylene poisoning. AMA Arch. Ind. Hyg. Occup. Med. 10:130-133, 1954.

McConnell, G., D. M. Ferguson, and C. R. Pearson. Chlorinated hydrocarbons and the environment. Endeavor 34:13-18, 1975.

McNally, W. D. A case of phosgene poisoning due to the inhalation of decomposition products of trichloroethylene. Ind. Med. 6:539-544, 1937.

McNeill, W. C. Chlorocarbons and Chlorohydrocarbons. Trichloroethylene. In: Kirk-Othmer's Encyclopedia of Chemical Technology. Volume 5. Third Edition, 1979. pp. 745-753.

Metz, L., and A. Roedy. Chem. Ing. Technile. 21:191, 1949. Cited in: Kirk-Othmer's Encyclopedia of Chemical Technology. Volume 5. Third Edition, 1979.

Migdal, A., Graczyk, E., Obodecka, Z., and K. Piesiak. Biochemical and neurological studies in acute oral poisoning with trichloroethylene. Wiad. Lek. 24:1669-1673, 1971.

Mikiskova, H., and A. Mikiska. Trichloroethanol in trichloroethylene poisoning. Br. J. Ind. Med. 23:116-125, 1966.

Mitchell, A. B. S., and B. C. Parsons-Smith. Trichloroethylene neuropathy. Br. Med. J. 1:422-423, 1969.

Mondino, A. Examination of the live mutant activity of the trichloroethylene compound with "Schizosaccharomyces pombe." Unpublished. 1979.

Monster, A. C., and G. Boersma. Simultaneous determination of trichloroethylene and metabolites in blood and exhaled air by gas chromatography. Int. Arch. Occup. Environ. Health 35:155-163, 1975.

Monster, A. C., G. Boersma, and W. C. Duba. Pharmacokinetics of trichloroethylene in volunteers; Influence of workload and exposure concentration. Int. Arch. Occup. Environ. Health 38:87-102, 1976.

Monster, A. C., G. Boersma, and W. C. Duba. Kinetics of trichloroethylene in repeated exposure of volunteers. Int. Arch. Occup. Environ. Health 42:283-292, 1979.

Morgan, A., A. Black, and D. R. Belcher. The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann. Occup. Hyg. 13:219-233, 1970.

Moslen, M. T., E. S. Reynolds, and S. Szabo. Enhancement of the metabolism and hepatotoxicity of trichloroethylene and perchloroethylene. Biochem. Pharmacol. 26:369, 1977.

Moslen, M. T., E. S. Reynolds, P. J. Boor, K. Bailey, and S. Szabo. Trichloroethylene-induced deactivation of P-450 and loss of liver glutathione *in vivo*. Res. Comm. Chem. Path. Pharmacol. 16:109-120, 1977.

Mroczek, H., and T. Fedyk. Przpadek ciezkiego, samobojczego zatrucia trojechloroetylenem ("Tri"). Pol. Tyg. Led. 26:1509-1510, 1971.

Muller, G., M. Spassovski, and D. Henschler. Metabolism of trichloroethylene in man. III. Interaction of trichloroethylene and ethanol. Arch. Toxicol. 33:173-189, 1975.

Muller, G., M. Spassovski, and D. Henschler. Trichloroethylene exposure, and trichloroethylene metabolites in urine and blood. Arch. Toxicol. 29:335-340, 1972.

Muller, G., M. Spassovski, and D. Henschler. Metabolism of trichloroethylene in man. II. Pharmacokinetics of metabolites. Arch. Toxic. 32:283-295, 1974.

Murray, A. J., and J. P. Riley. Occurrence of some chlorinated aliphatic hydrocarbons in the environment. Nature 242:37-38, 1973a.

Murray, A. J., and J. P. Riley. The determination of chlorinated aliphatic hydrocarbons in air, natural waters, marine organisms, and sediments. Anal. Chim. Acta 65:261-270, 1973b.

Nakanishi, S., E. Shiohara, M. Tsukada, H. Yamazaki, and K. Okumura. Acetaldehyde level in the blood and liver aldehyde dehydrogenase activities in trichloroethylene-treated rats. Arch. Toxicol. 41:207-214, 1978.

NAS (National Academy of Sciences). Drinking Water and Health. Safe Drinking Water Committee, Advisory Center on Toxicology, Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington, DC, p. 939. Available from: Printing and Publishing Office, National Academy of Sciences, 2101 Constitution Ave., Washington, DC 20418, 1977.

National Academy of Science. Nonfluorinated halomethanes in the environment. Panel on low molecular weight-halogenated hydrocarbons. Coordinating committee for scientific and technical assessments of environmental pollutants, 1978.

National Academy of Science. Ozone and Other Photochemical Oxidants. Committee on Medical and Biologic Effects of Environmental Pollutants, 1977.

National Bureau of Standards. Reaction Rate and Photochemical Data for Atmospheric Chemistry-1977. R.F. Hampson, Jr. and D. Garvin, eds., May 1978.

National Institute of Occupational Safety and Health. Occupational Exposure to Phosgene. HEW Publication No. 76-137, 1976.

NCI. Carcinogenesis bioassay of trichloroethylene. National Cancer Institute Carcinogenesis Technical Report Series No. 2, HEW Publication No. NIH 76-802, February 1976. 197 pp.

NIOSH, National Occupational Hazard Survey, Vol. III, Survey Analyses and Supplemental Tables, Cincinnati, USDHEW, pp. 2864-2866, 1977.

NIOSH. Registry of Toxic Effects of Chemical Substances, 1980 update.

Nomiyama, K. Estimation of trichloroethylene exposure by biological materials. Intl. Arch. Arbeitsmed. 27:281-292, 1971.

Nomiyama, K., and H. Nomiyama. Dose-response relationship for trichloroethylene in man. Int. Arch. Occup. Environ. Hlth. 39:237-248, 1977.

Nomiyama, K., and H. Nomiyama. Metabolism of trichloroethylene in humans. Sex difference in urinary excretion of trichloroacetic acid and trichloroethanol. Intl. Arch. Arbeitsmed. 28:37-48, 1971.

Nomiyama, K., and H. Nomiyama. Respiratory retention, uptake and excretion of organic solvents in man. Int. Arch. Arbeitsmed. 32:75-83, 1974a.

Nomiyama, K., and H. Nomiyama. Respiratory elimination of organic solvents in man. Int. Arch. Arbeitsmed. 32:85-91, 1974b.

Nomiyama, K., and H. Nomiyama. Trichloroethylene metabolism in man and animals, with special reference to host and agent factors modifying the trichloroethylene metabolism. Ind. Environ. Xenobiot. 2:173-176, 1977.

Noweir, M., E. A. Pfitzer, and T. F. Hatch. Decomposition of chlorinated hydrocarbons. A review. Amer. Ind. Hyg. Assoc. 33:454-460, 1973.

Nowill, W. K., C. R. Stephen, and G. Margolis. The chronic toxicity of trichloroethylene: a study. Anesthesiology 15:462-465, 1954.

NTP Technical Bulletin, (December, 1980) 1(3):8.

Oesch, F. Mammalian epoxide hydrases. Inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica, 3:305-340, 1973.

Oesch, F., N. Kaubisch, D. M. Jerina, and J. M. Daly. Hepatic epoxide hydrase. Structure-activity relationships for substrates and inhibitors. Biochem. 10:4858-4866, 1971.

Ogata, M., Y. Takatsuka, and K. Tomokuni. Excretion of organic chlorine compounds in the urine of persons exposed to vapours of trichloroethylene and tetrachloroethylene. Br. J. Ind. Med. 28:386-391, 1971.

Orth, O. S., and N. A. Gillespie. A further study of trichloroethylene anesthesia. Brit. J. Anaesth. 19:161-173, 1945.

Owens, A. H., and E. K. Marshall. Further studies on the metabolic fate of chloral hydrate and trichloroethanol. Bull. Johns Hopkins Hosp. 97:320-326, 1955.

Page, N. P., and Jack L. Arthur. Special Occupational Hazard Review of Trichloroethylene. DHEW 78-130, U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, Washington. DC. 1978.

Pasquini, D. A. Evaluation of glass sampling tubes for industrial breath analysis. Amer. Ind. Hyg. Assoc. 39:55-62, 1978.

Patty, F. A. Industrial Hygiene and Toxicology. 3 volumes. 2nd Revised Edition. Interscience Publishers, New York, NY, 1958.

Paykoc, Z. V., and J. F. Powell. The excretion of sodium trichloroacetate. J. Pharmacol. Exptl. Therap. 85:289-293, 1945.

PDR, Physician's Desk Reference, Oradell, N.Y.: Medical Economics Co., 1981. p. 1258.

Pearson, C. R., and G. McConnell. Chlorinated C_1 and C_2 hydrocarbons in the marine environment. Proc. Roy. Soc. London B. 189:305-332, 1975.

Pelkonen, O., and H. Vainio. Spectral interactions of a series of chlorinated hydrocarbons with cytochrome P-450 of liver microsomes from variously-treated rats. FEBS Lett. 51:11, 1975.

Pellizzari, E.D. Electron capture detection in gas chromatography. J. Chromatogr. 98:323-361, 1974.

Pellizzari, E. D. Development of Analytical Techniques for Measuring Ambient Atmospheric Carcinogenic Vapors EPA-600/2-75-075, November 1975.

Pellizzari, E. D., J. E. Bunch, R. E. Berkley and J. McRae. Collection and analysis of trace organic vapor pollutants in ambient atmospheres. The performance of Tenax GC cartridge sampler for hazardous vapors. Anal. Lett. 9:45-63, 1976.

Pellizzari, E. D. The Measurement of Carcinogenic Vapors in Ambient Atmospheres. EPA-600/7-77-055, June 1977.

Pellizzari, E. D., M. D. Erickson, and R. A. Zweidinger. Formulation of a Preliminary Assessment of Halogenated Organic Compounds in Man and Environmental Media. EPA-560/13-79-006, U.S. Environmental Protection Agency, July 1979.

Persson, H. On trichloroethylene intoxication. Acta Med. Scand. 59:410-422, 1934.

Pessayre, D., H. Allemand, J. C. Wandscheer, V. Descatoire, J-Y Artigou, and J-P. Benhamou. Inhibition, activation, destruction and induction of drug-metabolizing enzymes by trichloroethylene. Toxicol. Appl. Pharmacol. 49:355-363, 1979.

Pfaffenberger, C. D., A. J. Peoples, and H. E. Enos. Distribution of Volatile Halogenated Organic Compounds Between Rat Blood Serum and Adipose Tissue. Intern. J. Environ Anal. Chem., 1980.

Pfaffli, P., and A-L. Blackman. Trichloroethylene concentration in blood and expired air as indicators of occupational exposure. A preliminary report. Work-Environ-Health 9:140-144, 1972.

Piet, G. J., P. Slingerland, E. E. deGrunt, M. P. M. V. d. Heuvel, and B. C. J. Zoeteman. Determination of very volatile halogenated organic compounds in water by means of direct headspace analysis. Anal. Lett. A11:437-448, 1978.

Plessner, W. On trigeminal disease due to trichloroethylene intoxication. Neurol. Zentr. 34:916-918, 1915.

Powell, J. F. Trichoroethylene: absorption, elimination and metabolism. Brit. J. Ind. Med. 2:142-145, 1945.

Powell, J. F. Solubility or distribution coefficient of trichloroethylene in water, whole blood, and plasma. Brit. J. Indust. Med. 4:233-236, 1947.

Prendergast, J. A., R. A. Jones, L. J. Jenkins, Jr., and J. Siegel. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl. Pharmacol. 10:270-289, 1967.

Price, P. J., C. M. Hassett, and J. I. Mansfield. Transforming activities of trichloroethylene proposed and industrial alternatives. In Vitro 14:290-293, 1978.

Quadland, H. P. Petroleum solvents and trichloroethylene--Part 3 of the literature study of reports of occupational diseases attributed to volatile solvents. Ind. Med. 13:45-50, 1944.

Rall, D. P. Difficulties in extrapolating the results of toxicity studies in laboratory animals to man. Environ. Res. 2:360-367, 1969.

Rasmussen, R. A., D. E. Harsch, P. H. Sweany, J. P. Krasnec, and D. R. Cronn. Determination of atmospheric halocarbons by a temperature-programmed gas chromatographic freezeout concentration method. J. Air Pollut. Control Assoc. 27:579-581, 1977.

Reinhardt, C. F., L. S. Muller, and M. E. Maxfield. Epinephrine induced cardiac arrhythmia potential of some common industrial solvents. J. Occup. Med. 15:953, 1973.

Reinl, W. Scleroderma under the influence of trichloroethylene. Zentralbl. Arbeitsmed. 7:58-60, 1957.

Renberg, L. Determination of volatile halogenated hydrocarbons in water with XAD-4 resin. Anal. Chem. 50:1836-1838, 1978.

Reynolds, E. A return to trichloroethylene. Br. J. Anaesth. 48:51 (Letter), 1976.

Reynolds, E. S., and M. T. Moslen. Damage to hepatic cellular membranes by chlorinated olefins with emphasis on synergism and antagonism. Environ. Health Persp. 21:137-147, 1977.

Russell, J. W., and L. A. Shadoff. The sampling and determination of holocarbons in ambient air using concentration on porous polymer. J. Chromatogr. 134:275-284, 1977.

Salvini, M., S. Binaschi, and M. Riva. Evaluation of the psychophysiological functions in humans exposed to trichloroethylene. Br. J. Ind. Med. 28:293-295, 1971.

Sanders, V. M., A. N. Tucker, P. H. Hallett, B. Kaufman, R. A. Carchman, and A. E. Munson. Immunotoxicology of trichloroethylene in the mouse. II. Effects on humoral and cell-mediated immunity. Unpublished. 1980.

Sato, A., and T. Nakajima. A structure-activity relationship of chlorinated hydrocarbons. Arch. Environ. Health 34:69-75, 1979.

Sato, A., and T. Nakajima. Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. Brit. J. Ind. Med. 35:43-49, 1978.

Sato, A., T. Nakajima, Y. Fujiwara, and N. Murayama. A pharmacokinetic model to study the excretion of trichloroethylene and its metabolites after an inhalation exposure. Brit. J. Ind. Med. 34:55-63, 1977.

Schairer, L. A., J. Van't Hof, C. G. Hayes, R. M. Burton, and F. J. deSerres. Measurement of biological activity of ambient air mixtures using a mobile laboratory for in situ exposures. Preliminary results from the Tradescantia plant test system. EPA Pub. 600/9-78-027: 421-440, 1978.

Scherb, K. Studies on the evaporation of several low-molecular weight chlorinated hydrocarbons from a flowing channel. Muench eeiter. abwasser-fisch-flussboil. 30:235-248, 1978.

Schlossberg, L. Letter to Paul Price, U.S. Environmental Protection Agency, Washington, DC, June 26, 1980.

Schwetz, B. A., B. K. J. Leong, and P. J. Gehring. The effect of maternally inhaled trichloroethylene, perchloroethylene, methylchloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol. Appl. Pharmacol. 32:84-96, 1975.

Seage, A. J., and M. W. Burns. Pulmonary oedema following exposure to trichloroethylene. Med. J. Aust. 2:484-486, 1971.

Secchi, G. C., G. Chiappino, A. Lotto, and N. Zurlo. Actual chemical composition of commercial trilene and their hepatotoxic effects: clinical enzymologic study. Med. Lav. 59:486-497, 1968.

Seifter, J. Liver injury in dogs exposed to trichloroethylene. J Ind. Hyg. Toxicol. 26:250-252, 1944.

Sellers, E. M., and J. Koch-Weser. Potentiation of Warfarin-induced hypoprothrombinemia by chloral hydrate. N. Eng. J. Med. 283:827-831, 1970.

Sellers, E. M., G. Carr, J. G. Bernstein, S. Sellers, and J. Koch-Weser. Interaction of chloral hydrate and ethanol in man. II. Hemodynamics and performance. Clin. Pharmacol. Ther. 13:50-58, 1972a.

Sellers, E. M., M. Lang, J. Koch-Weser, E. LeBlanc, and H. Kalant. Interaction of chloral hydrate and ethanol in man. I. Metabolism. Clin. Pharmacol. & Therap. 13:37-49, 1972b.

Seto, T. A., and M. O. Schultze. Determination of trichloroethylene, trichloroacetic acid, and trichloroethanol in urine. Analyt. Chem. 28:1625-1629, 1956.

Shahin, M. M., and R. C. VonBorstel. Mutagenic and lethal effects of benzene hexachloride, dibutyl phthalate and trichloroethylene in *Saccharomyces cerevisiae*. Mutat. Res. 48:173-180, 1977.

Silverman, A. P., and H. Williams. Behaviour of rats exposed to trichloroethylene vapours. Br. J. Ind. Med. 32:308-315, 1975.

Simmon, V. F., K. Kauhanen, and R. G. Tardiff. Mutagenic activity of chemicals identified in drinking water. Pages 249-258 in D. Scott, B.A. Bridges and F.H. Solves, eds. Progress in genetic toxicology. Elsevier/North Holland Biomedical Press, 1977.

Singh, H. B. Atmospheric halocarbons: Evidence in favor of reduced average hydroxyl radical concentration in the troposphere. Geophy. Res. Lett. 4:101-104, 1977.

Singh, H. B. Discussions concerning trichloroethylene. Atmos. Environ. 12:1809, 1978.

Singh, H. B. Phosgene in the ambient air. Nature 264:428-429, 1976.

Singh, H. B., L. Salas, D. Lillian, R. R. Arnts, and A. Appleby. Generation of accurate halocarbon primary standards with permeation tubes. Environ. Sci. Technol. 11:511-513, 1977.

Singh, H. B., L. J. Salas, H. Shigeishi, A. J. Smith, E. Scribner, and L. A. Cavanagh. Atmospheric Distributions, Sources, and Sinks of Selected Halocarbons, Hydrocarbons, SF$_6$, and N$_2$O. EPA-600/3-79-107, U.S. Environmental Protection Agency, November 1979.

Singh, H. B., L. J. Salas, A. J. Smith, and H. Shigeishi. Measurement of some potentially hazardous organic chemicals in urban environments. Atmos. Environ. 15:601-612, 1981.

Sjöberg, B. Thermal decomposition of chlorinated hydrocarbons. Svensk. Kem. Tid. 64:63-79, 1952.

Skalko, R. G., and D. S. Packard, Jr. The incorporation of teratogenic agents into the mammalian embryo. IN: Congenital Defects, Janerich, et al. Eds., Academic Press, New York, pp: 235-254, 1974.

Slacik-Erben, R., R. Roll, G. Franke, and H. Uehleke. Trichloroethylene vapours do not produce dominant lethal mutations in male mice. Arch. Toxicol 45:37-44, 1980.

Smith, G. F. Trichloroethylene. A Review. Brit. J. Ind. Med. 23:249-262, 1966.

Smith, J. H., D. C. Bomberger, Jr., and D. L. Haynes. Prediction of the volatilization rates of high-volatility chemicals from natural water bodies. Environ. Sci. Technol. 14:1332-1337, 1980.

Soucek, B., and D. Vlachova. Excretion of trichloroethylene metabolites in human urine. Brit. J. Ind. Med. 17:60-64, 1960.

SRI International. Directory of Chemical Producers, 1978.

SRI International. In: Chemical Economic Handbook. Current Indicator Supplemental Data 632.3001J; 632.3001V; 632.3001W, Menlo Park, CA, 1981.

St. Hill, C. A. Occupation as a cause of sudden death. Trans. Soc. Occup. Med. 16:6-9, 1966.

Stewart, R. D., C. L. Hake, and J. E. Peterson. Use of breath analysis to monitor trichloroethylene exposures. Arch. Environ. Health 29:6-13, 1974.

Stewart, R. D., H. C. Dodd, H. H. Gay, and D. S. Erley. Experimental human exposure to trichloroethylene. Arch. Environ. Health 20:64-71, 1970.

Stewart, R. D., H. H. Gay, D. S. Erley, C. L. Hake, and J. E. Peterson. Observations on the concentrations of trichloroethylene in blood and expired air following exposure of humans. Am. Ind. Hyg. Assoc. J. 23:167-172, 1962.

Stewart, R. D., and H. C. Dodd. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin. Am. Ind. Hyg. Assoc. J. 25:439-446, 1964.

Stewart, R. D., C. L. Hake, and J. E. Peterson. "Degreasers' flush": dermal response to trichloroethylene and ethanol. Arch. Environ. Hlth. 29:1-5, 1974.

Stopps, G. J., and M. McLaughlin. Psychophysiological testing of human subjects exposed to solvent vapors. Am. Ind. Hyg. Assoc. J. 28:43-50, 1967.

Stott, W. T., J. F. Quast, and P. G. Watanabe. The Pharmacokinetics and Macromolecular Interactions of Trichloroethylene in Mice and Rats as Related to Oncogenicity. Prepared for Chemical Manufactures Association. Washington, DC. Ref. No. TCE 2.0-PHKS/MACM. 33 pp.

Striker, C., S. Goldblatt, I. S. Wann, and D. E. Jackson. Clinical experience with use of trichloroethylene in production of over 300 analgesias and anesthesias. Anesth. and Analg. 14:68-71, 1935.

Stuber, K. Gesundheitsschadigen bei der Gewerblichen Verwendung des Trichloroalthylens und die Monglichkelien ihrer Verhutung. Arch. Gewerbepath. Bewerbehyg. 2:398-456, 1931.

Su, G., and K. A. Wurzel. A regulatory framework for setting air emission limits for noncriteria pollutants. J. Air Pollut. Contr. Assoc. 31(2): 160-162, 1981.

Styles, J. A. Cell transformation assays. In: Topics in Toxicology: Mutagenesis in Sub-mammalian Systems: Status and Significance. C. E. Paget, Ed. University Park Press Baltimore, 1979. pp. 147-164.

Tada, T., T. Ohta, and I. Mizoguchi. Behavior of chlorinated hydrocarbons in urban air. Ann. Rep. Tokyo Metr. Res. Lab. P.H. 27:242-246, 1976.

Tanaka, S., and M. Ikeda. Method for determination of trichloroethanol and trichloroacetic acid in urine. Brit. J. Ind. Med. 25:214-219, 1968.

Taylor, H. Experiments on the physiological properties of trichloroethylene. J. Ind. Hyg. Toxicol. 18:175-193, 1936.

Tham, R., B. Larsby, L. Odkvist, B. Norlander, D. Hyden, G. Aschan, and A. Bertler. The influence of trichloroethylene and related drugs on the vestibular system. Acta Pharmacol. Toxicol. 44:336-342, 1979.

Toftgard, R., and J-A Gustafsson. Biotransformation of organic solvents: A review. Scand. J. Work Environ. Health 6:1-18, 1980.

Tola, J. As cited by Page, N.P. and J.L. Arthur (eds.). In: Special Occupational Hazard Review of Trichloroethylene. 79 pp. DHEW NIOSH 78-130, 1978.

Tolot, F., J. Viallier, A. Roullett, J. Rivoire, and J.C. Figueres. Hepatic toxicity of trichloroethylene. Arch. Mal. Prof. Med. Trav. Secur. Soc. 25:9-15, 1964.

Tomasini, M., and E. Sartorelli. Chronic intoxication from commercial trielin inhalation with compromise of the eighth cranial nerves. Med. Lav. 62:277-280, 1971.

Tomatis, L. Report of Discussion Group No. 4. Inception and duration of tests. In: Carcinogenesis Testing of Chemicals, L. Golberg, ed., CRC Press, Cleveland, OH., 1974. pp. 23-27.

Tottmar, S. O. C., H. Petterson, and K. H. Kiessling. The subcellular distribution and properties of aldehyde dehydrogenase in rat liver. Biochem. J. 135:577-586, 1973.

Traylor, P. S., W. Nastainczyk, and V. Ullrich. Conversion of trichloroethylene to carbon monoxide by microsomal cytochrome P_{450}. *In*: Microsomes and drug oxidations. Proceedings of the 3rd International Symposium, Berlin, 1976. Suppl. Biochem. Pharmacol., Pergamon, Oxford, 1977. pp. 615-621.

Tsuruta, H. Percutaneous absorption of trichloroethylene in mice. Ind. Health 16:145, 1978.

Tucker, A. N. M., V. M. Sanders, D. W. Barnes, I. J. Bradshaw, and A. E. Munson. Immunotoxicology of trichloroethylene in the mouse. I. General toxicology. Unpublished. 1980.

Uehleke, H., and S. Poplawski-Tabarelli. Irreversible binding of ^{14}C-labelled trichloroethylene to mice liver constituents *in vivo* and *in vitro*. Arch. Toxicol. 37:289-294, 1977.

Uehleke, H., S. Poplawski, G. Bonse, and D. Henschler. Spectral evidence for 2,2,3-trichloro-oxirane formation during microsomal trichloroethylene oxidation. Naunyn-Schmiedebergs Arch. Pharmacol. 293:(Suppl.) 253 (abst), 1976a.

Uehleke, H., S. Poplawski, G. Bonse, and D. Henschler. Spectral evidence for oxirane formation during microsomal trichloroethylene oxidation. Arch. Toxicol. 37:95-105, 1976b.

U.S. Environmental Protection Agency. Air Quality Criteria for Ozone and Other Photochemical Oxidants. EPA-600/8-78-004, April 1978, pp. 5-52.

U. S. Environmental Protection Agency. Ambient Water Quality Criteria for Halomethanes. U. S. EPA. Available from: National Technical Information Services, Springfield, VA (NTIS PB81-117624), 1980b.

U.S. Environmental Protection Agency. Ambient Water Quality Criteria: Trichloroethylene. EPA-440/5-80-077, October, 1980.

U.S. Environmental Protection Agency. An Assessment of the Need for Limitations on Trichloroethylene, Methyl Chloroform, and Perchloroethylene. EPA-560/11-79-009, July 1979.

U.S. Environmental Protection Agency. Draft Exposure Assessment for TSPC Solvents. TSPC Solvents Work Group No. 2, July 1981.

U.S. Environmental Protection Agency. Exposure Assessment: The Occurrence of Volatile Organic Chemicals in Drinking Water. Office of Drinking Water, Washington, DC, March 1981.

U.S. Environmental Protection Agency. Guidelines and Methodology for the Preparation of Health Effect Assessment Chapters of the Ambient Water Quality Criteria Documents. U. S. EPA, Environmental Criteria and Assessment Office; Office of Health and Environmental Assessment; Office of Research and Development, Cincinnati, OH, November 28, 1980a.

U.S. Environmental Protection Agency. In-Depth Studies on Health and Environmental Impacts of Selected Water Pollutants. Contract No. 68-01-4646, Duluth, MN, 1978.

U.S. Environmental Protection Agency. Preliminary Study of Selected Potential Environmental Contaminants-Optical Brighteners, Methyl Chloroform, Trichloroethylene, Tetrachloroethylene, Ion Exchange Resins. EPA-560/2-75-002, July 1975.

U.S. International Trade Commission. Synthetic Organic Chemicals. United States Production and Sales. USITC Publication 920, 1978.

U.S. Occupational Safety and Health Administration. Air contaminants. US Code Fed. Regl., Title 29, part 1910, 1000, pp. 612-614, 1978.

Utesch, R. C., F. W. Weir, and J. V. Bruckner. Development of an animal model of solvent abuse for use in evaluation of extreme trichloroethylene inhalation. Toxicology 19:169-182, 1981.

Van Duuren, B. L. Chemical structure, reactivity and carcinogenicity of halohydrocarbons. Environ. Health Persp. 21:17-23, 1977.

Van Duuren, B. L., and S. Banerjee. Covalent interaction of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res. 36:2419-2422, 1976.

Van Duuren, B. L., B. M. Goldschmidt, G. Lowengart, A. C. Smith, S. Melchionne, I. Seldman, and D. Roth. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. J. Natl. Cancer Inst. 63:1433-1439, 1979.

Van Dyke, R. Dechlorination mechanisms of chlorinated olefins. Environ. Health Persp. 21:121-124, 1977.

Van Dyke, R., and M. B. Chenoweth. Metabolism of volatile anesthetics. Anesth. 26:348, 1965.

Vernon, R. J., and R. K. Ferguson. Effects of trichloroethylene on visual-motor performance. Arch. Environ. Hlth. 18:894-900, 1969.

Vesterburg, O., and I. Astrand. Exposure to trichloroethylene monitored by analysis of metabolites in blood and urine. J. Occup. Med. 18:224-226, 1976.

Vesterburg, O., J. Gorczak, and M. Krasts. Exposure to trichloroethylene. II. Metabolites in blood and urine. Scand. J. Work Environ. & Health 4:212-219, 1976.

Vignoli, L., Jouglard, J., Vignoli, P., and Terrasson de Fougeres, P. Acute intoxication by trichloroethylene ingestion. Med. Leg. Assicur. 18:789-798, 1970.

Viola, P. L., A. Bigotti, and A. Caputi. Oncogenic response of rat skin, lungs, and bones to vinyl chloride. Cancer Res. 31:516-522, 1971.

Vyskocil, J., and B. Polak. Acute trichloroethylene poisoning. Vnitr. Lek. 9:860-863, 1963.

Waskell, L. A study of the mutagenicity of anesthetics and their metabolites. Mutat. Res. 57:141-153, 1978.

Waters, E. M., H. B. Gerstner, and J. E. Huff. Trichloroethylene. I. An overview. J. Toxicol. Environ. Health 2:671-707, 1977.

Waxweiler, R. J., et al. Neoplastic risk among workers exposed to vinyl chloride. Ann. NY Acad. Sci. 271:40-48, 1976.

Werch, S. C., G. H. Marquardt, and J. F. Mallach. The action of trichloroethylene on the cardiovascular system. Am. J. Obst. and Gyn. 69:352-364, 1955.

Withey, J. R., and B. T. Collins. Chlorinated aliphatic hydrocarbons used in the foods industry: The comparative pharmacokinetics of methylene chloride, 1,2-dichloroethane, chloroform, and trichloroethylene after i.v. administration in the rat. J. Environ. Pathol. Toxicol. 3:313-332, 1980.

White, A. S., S. Takehisa, E. I. Eger II., S. Wolff, and W. C. Stevens. Sister-chromatid exchanges induced by inhaled anesthetics. Anesthesiology 50:426-430, 1979.

Whitteridge, D., and E. Bülbring. Changes in activity of pulmonary receptors in anesthesia and their influence on respiratory behaviour. J. Pharmacol. Exptl. Therap. 81:340-359, 1944.

Wiecko, W. Oral poisoning with trichloroethylene. Wiad. Lek. 19:1117-1118, 1966.

Wirtschafter, Z. T., and M. W. Cronyn. Relative hepatotoxicity. Arch Environ. Hlth. 9:180-185, 1964.

World Health Organization. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, No. 4, Geneva, April 4-12, 1972.

Yacoub, M., J. Faure, R. Rollux, J. Mallion, and C. Marka. L'intoxication aigue par le trichloroethylene. Manifestations cliniques et paracliniques, surveillance de l'elimination du produit. J. Eur. Toxicol. 6:275-283, 1973.

York et al. The Toxicologist 1(1):28 (abstract), 1981.

Zoeteman, B. C. J., K. Harmsen, J. B. H. J. Linders, C. E. H. Morra, and W. Sloof. Persistent organic pollutants in river water and ground water of the Netherlands. Chemosphere 9:231-249, 1980.

EXTERNAL REVIEW DRAFT
November 20, 1981

THE CARCINOGEN ASSESSMENT GROUP'S
CARCINOGEN ASSESSMENT
OF
TRICHLOROETHYLENE

Roy E. Albert, M.D.
Chairman

PARTICIPATING MEMBERS

Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.
Margaret M.L. Chu, Ph.D.
Herman J. Gibb, B.S., M.P.H.
John R. Fowle III, Ph.D.*
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert McGaughy, Ph.D.
Dharm V. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

*Reproductive Effects Assessment Group

DRAFT
DO NOT QUOTE OR CITE

This document has been reviewed and approved by the Chairman and staff of the Carcinogen Assessment Group, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency. It has not been formally released by the EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implication.

CONTENTS

I. Summary and Conclusions. 1

  Qualitative Assessment
  Quantitative Assessment

II. Introduction 7

III. Metabolism 9

IV. Mutagenicity and Cell Transformation 38

  Testing of Technical Grade Material
  Testing of Purified Material
  Cell Transformation

V. Toxicity 79

VI. Carcinogenicity. 85

  Animal Data
  Contaminants of Technical Trichloroethylene
  Epidemiological Data

VII. Unit Risk Estimate 114

  Conversion of Units
  National Cancer Institute (1976) Cancer Bioassay in Mice
  Upper-Limit Calculation Based on the Axelson et al. (1978)
    Study in Humans

VIII. References 121

I. SUMMARY AND CONCLUSIONS

QUALITATIVE ASSESSMENT

Technical grade trichloroethylene, administered at maximally tolerated and one-half maximally tolerated dosages by gavage for 78 weeks, was shown to induce a statistically significant increase in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice; metastases were also found in males in both treatment groups. In a second lifetime study, in which mice were exposed to technical grade trichloroethylene vapor by inhalation for 24 months, a statistically significant increase in the incidence of hepatocellular carcinomas in male B6C3F1 mice was observed; however, this study is weakened by wide variability in the trichloroethylene exposure concentrations and the lack of concurrent matched controls. Two chronic rat studies with technical grade trichloroethylene, one with Osborne-Mendel rats by gavage and the other with Charles River rats by inhalation, showed no carcinogenic response. In a study of female Han:NMRI mice exposed to purified grade trichloroethylene vapor for 18 months, a statistically significant increase in the incidence of malignant lymphomas was found; trichloroethylene was not observed to be carcinogenic in Han:Wist rats, Syrian hamsters, and male Han:NMRI mice when dosed similarly. The B6C3F1 mice in the first two studies were treated with technical grade trichloroethylene contaminated with the epoxides epichlorohydrin and 1,2-epoxybutane, whereas the Han:NMRI mice, the Han:Wist rats, and the hamsters were exposed to purified grade trichloroethylene containing no detectable epoxides.

It is possible that the induction of malignant lymphomas in female Han:NMRI mice by purified grade trichloroethylene could have been due to an immunosuppressive effect of trichloroethylene; however, since little is known about the dose-response relationship for immunosuppressive agents, the significance of trichloroethylene exposure at doses considerably below those to which the Han:NMRI mice were exposed on immunosuppression is not clear.

Other chronic animal studies have been reported which indicate that trichloroethylene of undetermined purity, administered by gavage or inhalation, was not carcinogenic; however, duration of treatment was either less than the lifetime of the animals or, in one study, not specified. In lifetime studies of ICR/Ha Swiss mice treated with purified grade trichloroethylene at doses below that maximally tolerated, no carcinogenic response was observed via dermal application in females, or with once-weekly administration by gavage or subcutaneous injection.

Neither trichloroethylene nor trichloroethylene epoxide was found to have initiating activity in a lifetime initiation-promotion experiment in female ICR/Ha Swiss mice treated with the same initiating dose as that used in the repeated dosing studies, which were below the maximum tolerated dose. Trichloroethylene epoxide applied topically and subcutaneously in female ICR/Ha Swiss mice induced no carcinogenic response at the site of application, according to prepublication data; no indication of the maximum tolerated dose in these experiments is available.

Trichloroethylene was not observed to be carcinogenic in occupational epidemiology studies done in Sweden and Finland. The cohort in the Swedish study was small (518 workers), and the majority of workers was under 40 years of age and the latency period was 6 to 13 years in the Finnish study. Estimated exposure levels of trichloroethylene for the majority of workers in both studies were at or below 30 ppm; however, exposure durations were not known.

A number of studies have been conducted to assess the mutagenic potential of trichloroethylene, including primary DNA damage, chromosomal aberrations, gene mutation, alterations in cell growth and mitosis, and ability of the compound to reach the gonads of an intact mammal. The results of these studies have been conflicting, but because of trichloroethylene's high volatility and low solubility, it has been difficult to adequately test this material. Several investigators reporting negative results possibly failed to take these properties into account and the concern is raised that the target cells were not adequately exposed. Furthermore, trichloroethylene is unstable and the chemical substances which have been employed to stabilize it have often been potent mutagens. Therefore, positive results reported in studies using technical grade samples known to contain mutagenic stabilizers or reports of studies which do not contain information concerning the purity of the tested material should be viewed with caution. In such cases it is not possible to ascribe any mutagenic response observed exclusively to trichloroethylene. Concerning the testing of technical grade trichloroethylene for mutagenicity, positive responses were observed with the base pair substitution-detecting *Salmonella typhimurium* strains TA 100 and TA 1535 and the *Saccharomyces cerevisiae* XV 185-14C haploid strain both with and without metabolic activation. Similarly, it was reported that human exposure to technical grade trichloroethylene results in an increase in hypodiploid cells. It has also been reported that technical grade trichloroethylene causes somatic cell mutations in *Tradescantia* and alters cell growth and mitosis in Chinese hamster fibroblast cells and *Euglena*. Negative results have been reported after testing technical grade trichloroethylene in *S. typhimurium* strain TA 100 with metabolic activation, in *Schizosaccharomyces pombe* host-mediated assays in mice, and in a *Drosophila melanogaster* sex-linked recessive lethal test. Purified (analytical) grade trichloroethylene produced weak (less than twofold) mutagenic responses in the presence of metabolic activation in three tests conducted using *S. typhimurium* strain TA 100 and three tests using _S. cerevisiae_ strains D4 and D7, and induced cell transformation _in vitro_ in a Fischer F1706 rat embryo cell line. Cell transformation was not found in baby Syrian hamster kidney (BHK-21/Cl 13) cells exposed to liquid unstabilized trichloroethylene _in vitro_. Negative responses were reported in two tests conducted with _Salmonella_ strains, one dominant lethal study employing mice, and one study of the ability of trichloroethylene to cause SCEs in Chinese hamster ovary cells. Evidence is available to indicate that trichloroethylene is metabolically converted to a reactive epoxide intermediate, and epoxides as a group are considered to be potentially carcinogenic. Chloral hydrate, a metabolite of trichloroethylene, was shown to be weakly mutagenic (less than twofold increase) in a _S. typhimurium_ TA 100/rat liver microsome assay. Based on the weight of evidence available at this time, it is concluded that trichloroethylene is a weak mutagen by virtue of its conversion to metabolic products which can interact with DNA.

In summary, evidence that technical grade trichloroethylene (epoxide-stabilized) has carcinogenic activity is based on the increased incidence of hepatocellular carcinomas in exposed B6C3F1 mice, the positive mutagenic responses in bacteria and yeast, and the positive mutagenic response of bacteria to chloral hydrate. Applying the International Agency for Research on Cancer (IARC) criteria for animal studies, this level of evidence would be regarded as limited and not sufficient to provide a firm conclusion on its carcinogenic potential in humans.

In addition, evidence that purified grade trichloroethylene has carcinogenic activity consists of the increased lymphoma incidence in Han:NMRI mice; the positive mutagenic responses in bacteria, yeast, and the mouse spot test in the presence of purified (analytical) grade trichloroethylene and in bacteria exposed to chloral hydrate; and the positive response in the _in vitro_ cell transformation study in Fischer F1706 rat embryo cells with purified (analytical) grade trichloroethylene. Applying the IARC criteria for animals, this level of evidence would be regarded as limited and not sufficient to provide a firm conclusion on its carcinogenic potential in humans. Furthermore, the relatively high spontaneous incidence of lymphoma and the possibility of an indirect mechanism of response in the Han:NMRI mice make the evidence of carcinogenicity for purified trichloroethylene weaker than for the technical material.

The carcinogenicity of trichloroethylene, devoid of detectable epoxides, is being evaluated by the National Toxicology Program (NTP) with gavage administration and by C. Maltoni and Associates via inhalation exposure in ongoing studies in various strains of mice and rats. The results of these studies should provide more definitive information about the carcinogenicity of purified trichloroethylene.

The Carcinogen Assessment Group recently examined the Preliminary Review of the National Toxicology Program's (NTP) Carcinogenesis Bioassay of Trichloroethylene, dated October 23, 1981 (NIH 82-1799).* This bioassay administered pure "Hi-Tri" trichloroethylene (free of epichlorohydrin stabilizers) in corn oil by gavage to groups of 50 male and female Fischer 344 rats at doses of 500 or 1000 mg/kg and two groups of 50 male and female B6C3F1 mice at doses of 1000 mg/kg, dosed five times per week for up to 103 weeks. This preliminary draft reported statistically significant incidences of renal carcinomas or adenocarcinomas in male F344 rats, statistically significant incidences of hepatocellular carcinomas in both male and female B6C3F1 mice, and the unusual occurrence of squamous-cell papillomas and a squamous-cell carcinoma of the stomach in the treated female mice. It should be noted that this preliminary report supports the carcinogenicity of pure trichloroethylene and that carcinogenicity occurred in two species, which would cause it to be categorized as having sufficient evidence of carcinogenicity by the IARC criteria. This study cannot be fully evaluated until it is released in its final form, which is expected to be sometime in June 1982.

---

*The following disclaimer was attached to this NTP report: "This is not a final report. Until this DRAFT is reviewed and approved by the Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors, this DRAFT does not represent the official position of the National Toxicology Program."

QUANTITATIVE ASSESSMENT

A unit risk estimate for purified grade trichloroethylene is being deferred until these additional studies are available for review. A unit risk estimate based on the lymphoma response in female Han:NMRI mice from inhalation exposure is not made because of the possibility that the lymphoma incidence in this study is due to an immunosuppressive effect of trichloroethylene and because of the questionable nature of the dose-response relationship for immunosuppressive agents. A unit risk estimate for technical grade trichloroethylene is calculated using the hepatocellular carcinoma response in male B6C3F1 mice given trichloroethylene by gavage for 78 weeks in the NCI (1976) bioassay. The linearized multistage model is used for low-dose extrapolation in order to give an estimate of a plausible upper bound of lifetime cancer risk, recognizing that uncertainties in both the qualitative evaluation and the quantitative extrapolation process give rise to a lower bound of zero risk. The plausibility of the upper bound is enhanced when there is direct evidence for genotoxicity, which is the case for trichloroethylene.

Using this procedure, the upper bound of the individual lifetime cancer risk resulting from continuous exposure to air with a trichloroethylene concentration of 1 ug/m^3 is 2.7×10^{-6}. The corresponding risk for drinking water concentration of 1 ug/l is 3.6×10^{-7}. Because of the uncertainties in both the qualitative and quantitative aspects of risk assessment, the actual quantitative cancer risks may be lower that those indicated above, which should be regarded as plausible upper-limits, and may approach zero.

Based on a negative epidemiologic study of Swedish factory workers, a 95% upper-limit estimate of risk is 1.7×10^{-5} for a continuous exposure to 1 ug trichloroethylene/m^3 air; however, this upper-limit calculation is based on total cancer deaths in this study rather than on an increase in cancer in a specific target tissue in the exposed workers.

II. INTRODUCTION

Trichloroethylene (1,1,2-trichloroethylene) is a volatile, nonflammable, colorless liquid with an odor resembling that of chloroform. Its structural formula is $Cl_2C = CHCl$, and it has a molecular weight of 131.4. Physical properties of trichloroethylene include a density of 1.4642, a boiling point of 86.7°C, a vapor pressure of 94 mm Hg at 30°C, slight solubility with water (110 mg/100 g water at 20°C), and ready solubility in organic solvents. Trichloroethylene reacts with sulfhydryl groups, slowly decomposes in light to produce hydrogen chloride, and is subject to autooxidation. Contact of trichloroethylene with hot metal or fire yields phosgene gas and hydrogen chloride. Dichloroethylene, which reacts with air to produce phosgene and carbon monoxide, may be formed from trichloroethylene in the presence of a strong alkali. Stabilizers commonly added to trichloroethylene include thymol or ammonium carbonate for the clinical grade and simple amines, epoxides, ethanolamine, aniline, or mixtures of organic compounds for the industrial grade (Waters et al. 1977, International Agency for Research on Cancer 1976).

Annual production of trichloroethylene in the United States was approximately 115,739 metric tons in 1979 (U.S. EPA 1980), and the estimated number of workers exposed to trichloroethylene on a full-time basis in the United States as of 1978 was 100,000 (Page and Arthur 1978). The major use of trichloroethylene in the United States is for vapor degreasing of fabricated metal parts. It is also used as a solvent in cleaning fluids, an inhalation anesthetic, and a chain terminator in polyvinyl chloride production (Waters et al. 1977). The threshold limit value in the United States is currently 100 ppm (535 mg/m^3) (National Institute for Occupational Safety and Health 1973).

Estimated ambient trichloroethylene concentrations at eight locations in five American states in 1974 generally exceeded 270 ng/m^3 (50 ppt) in urban areas but usually was less than 270 ng/m^3 (50 ppt) in rural areas (Lillian et al. 1975). Trichloroethylene levels above 1,000 ng/m^3 were determined in several air samples collected in various urban areas in New Jersey and in Niagara Falls and Staten Island, New York during 1976 to 1978 (U.S. EPA 1979). In a survey of trichloroethylene residues in various foodstuff, McConnell et al. (1975) found the greatest quantities to be in butter (10 ug/kg), meat products (16-22 ug/kg), and tea (60 ug/kg); however, use of trichloroethylene as an extractant in food processing was discontinued in the United States (Fishbein 1979).

III. METABOLISM

Figure 1 presents a proposed intermediary metabolic scheme for trichloroethylene by Waters et al. (1977). Bonse et al. (1975) found that trichloroethylene oxide chemically rearranges in nonpolar solvents to dichloroacetyl chloride, whereas, in contrast, trichloroethylene is metabolically converted by an isolated perfused rat liver preparation to chloral hydrate, trichloroacetic acid, and trichloroethanol and its glucuronide. However, Bonse and Henschler (1976) reported that a spontaneous chemical rearrangement of trichloroethylene oxide to chloral in nonpolar solvents could be forced with the addition of Lewis acids. Henschler and Bonse (1978) consequently postulated that since the intramolecular rearrangement of trichloroethylene oxide to trichloroacetaldehyde is apparently dependent on the presence of Lewis acids, the metabolic conversion of the epoxide shown in Figure 1 is feasible by virtue of the trivalent iron in microsomal cytochrome P450.

Irreversible binding of [1,2-^{14}C] trichloroethylene metabolites with microsomal protein and ribonucleic acid (RNA) was demonstrated on incubation of male Wistar rat liver microsomes, a NADPH-regenerating system and/or yeast RNA in the presence of radiolabeled trichloroethylene vapor at a partial pressure of $<$ 0.5 torr (substrate saturation conditions) for 60 minutes at 37°C. Amounts of covalently labeled metabolites were approximately 1.02 umol/mg protein and 21 pmol/mg RNA (Bolt et al. 1977, Laib et al. 1979). Similar binding of ^{14}C-trichloroethylene metabolites to mouse hepatic protein both _in vivo_ and _in vitro_ also was reported (Uehleke and Poplawski-Tabarelli 1977). Irreversible binding to liver microsomal proteins was determined after either single intraperitoneal or gavage administration of 0.2 uCi ^{14}C-trichloroethylene/g body weight into NMRI mice. Binding was greatest (5 to 8 nmol trichloroethylene/mg protein) at 6 hours after dosing with radioactivity and was

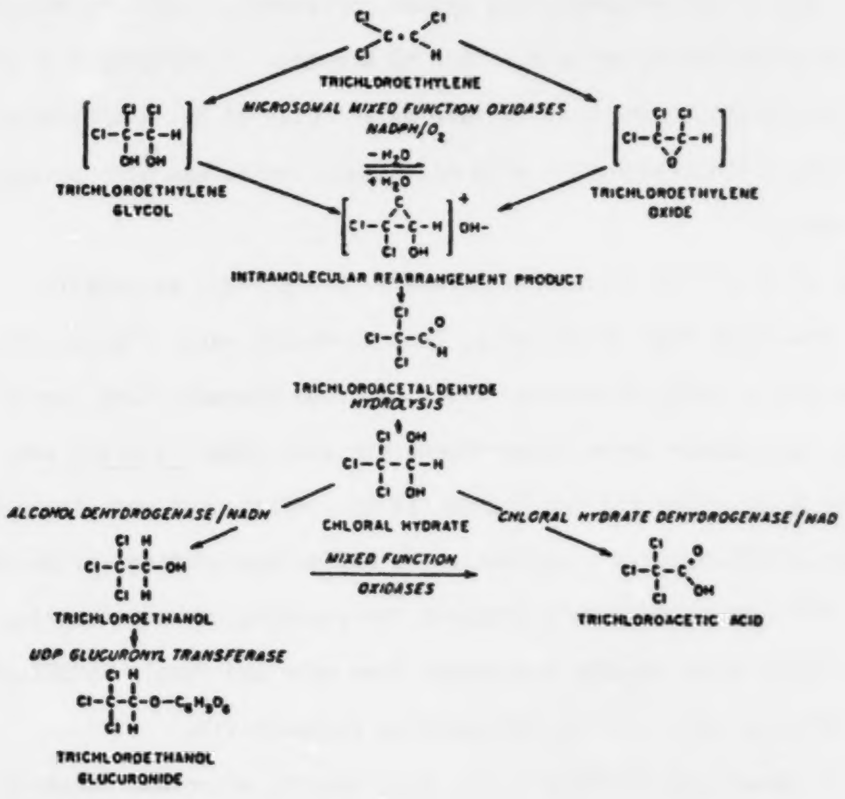

TRICHLOROETHYLENE
MICROSOMAL MIXED FUNCTION OXIDASES
NADPH/O2
-H2O
+H2O
TRICHLOROETHYLENE GLYCOL
TRICHLOROETHYLENE OXIDE
INTRAMOLECULAR REARRANGEMENT PRODUCT
TRICHLOROACETALDEHYDE
HYDROLYSIS
CHLORAL HYDRATE
ALCOHOL DEHYDROGENASE/NADH
CHLORAL HYDRATE DEHYDROGENASE/NAD
MIXED FUNCTION
OXIDASES
TRICHLOROETHANOL
TRICHLOROACETIC ACID
UDP GLUCURONYL TRANSFERASE
TRICHLOROETHANOL GLUCURONIDE

Figure 1. Proposed intermediary metabolism of trichloroethylene.
Waters et al. (1977)

still detectable at 24 hours. Irreversible binding of ^{14}C-trichloroethylene to nonmetabolizing mouse liver microsomes in vitro was also found; however, the trichloroethylene sample was only 89% pure. Purification of this product resulted in a 60 to 70% reduction in binding to nonmetabolizing microsomes. In an incubation system of the purified ^{14}C-trichloroethylene, mouse liver microsomes, and a NADPH-regenerating system, irreversible binding attained 1.4 umol trichloroethylene/mg protein within 60 minutes. Pretreatment of mice with phenobarbital increased the binding rate by a factor of 2. A similar pattern of binding of ^{14}C-trichloroethylene with microsomal lipids was also observed in the experiments.

Binding of [1,2-^{14}C] trichloroethylene (> 99% purity) to hepatic microsomal fractions from B6C3F1 mice, Osborne-Mendel rats, Fischer 344 rats, and Sprague-Dawley rats; microsomal fractions from stomach, lung, and kidney of B6C3F1 mice; and salmon sperm deoxyribonucleic acid (DNA) in vitro was investigated by Banerjee and Van Duuren (1978). Microsomes were incubated in the presence of ^{14}C-trichloroethylene and a NADPH-regenerating system for 60 minutes at 37°C and subsequently prepared for counting of radioactivity. The DNA was incubated with hepatic microsomes from male and female B6C3F1 mice under similar conditions and purified for counting radioactivity.

The data summarized in Table 1 show that hepatic microsomal binding of ^{14}C-trichloroethylene was greater in mice compared to rats and greater in males compared to females. Microsomes from other tissues of mice also effectively bound ^{14}C-trichloroethylene. Hepatic microsomes from male mice more effectively mediated binding of radioactivity to DNA than hepatic microsomes from female mice. Binding to DNA required the presence of microsomes. The interaction between ^{14}C-trichloroethylene and both microsomes and DNA was determined to be covalent in nature.

TABLE 1. BINDING OF^{14}C-TRICHLOROETHYLENE TO MICROSOMAL PROTEIN AND SALMON SPERM DEOXYRIBONUCLEIC ACID (DNA)

(adapted from Banerjee and Van Duuren 1978)

Species/Strain*	Tissue	umol ^{14}C-trichloroethylene/mg microsomal protein†		umol ^{14}C-trichloroethylene/mg DNA†	
		Male	Female	Male	Female
Mouse/B6C3F$_1$	Liver	2.6 ± 0.3	1.9 ± 0.1		
Rat/Sprague-Dawley	Liver	2.6 ± 0.2	No data		
Rat/Osborne-Mendel	Liver	1.8 ± 0.2	1.4 ± 0.1		
Rat/Fischer 344	Liver	1.6 ± 0.3	1.3 ± 0.2		
Mouse/B6C3F1	Stomach	3.0	2.7		
Mouse/B6C3F1	Lung	2.9	3.3		
Mouse/B6C3F1	Kidney	2.2	2.1		
Mouse B6C3F1	Liver			93.2 ± 11.2	35.7 ± 2.1

*Animals were 5 to 9 weeks of age.
†Mean ± S.E.

The following additional observations in this study suggest that trichloroethylene was bound to mouse and rat tissue microsomes and salmon sperm DNA as a reactive metabolic intermediate: 1) Pretreatment of male B6C3F1 mice with phenobarbital, an inducer of microsomal metabolic enzymes, increased *in vitro* ^{14}C-trichloroethylene binding to hepatic microsomes by 58% over control levels (2.6 ± 0.3 nmol/mg protein) and DNA by 41% over control levels (92.5 ± 5.0 pmol/mg DNA); 2) Addition of 1.2, 2.4, or 4.8 mM concentrations of 3,3,3-trichloropropene epoxide, an inhibitor of epoxide hydrase, to incubation systems containing hepatic microsomes from male B6C3F1 mice produced a dose-related increase in ^{14}C-trichloroethylene binding to DNA of 14, 41, and 120% above the control level (81 pmol/mg DNA) in contrast to the ^{14}C-trichloroethylene binding to microsomes which was increased by 15% with the low dose and decreased by 32 and 39% with the other doses compared to the control level of 2.4 nmol/mg protein.

Uehleke et al. (1977) have obtained spectral evidence for trichloroethylene oxide formation *in vitro*. Addition of nonstabilized trichloroethylene purified by distillation to a suspension of male rabbit liver microsomes in the presence of NADPH and aerobic conditions produced an absorption peak between 451 and 452 nm after incubation at 37°C for 5 minutes. Chloral and trichloroacetic acid were ineffective, and trichloroethanol yielded a broad absorption peak with a maximum at 480 nm and a relatively low value at 450 nm. Dichloroacetyl chloride, the main thermal rearrangement product of the assumed epoxide intermediate, produced an absorption peak at 452 nm which was approximately fivefold smaller than that formed from an equimolar amount of trichloroethylene. A concentration-related increase in the absorption peak at 451-452 nm was observed on addition of trichloroethylene oxide, with dichloroacetyl chloride removed, to microsomal suspensions containing either dithionite or NADPH as reducing agents; furthermore, the change in absorbance produced by dichloroacetyl chloride was only 0.2 of that caused by trichloroethylene oxide on an equimolar basis. Hence, the correspondence between absorption peaks elicited by trichloroethylene and trichloroethylene oxide provides evidence that the latter is formed during trichloroethylene metabolism.

Van Duuren and Banerjee (1976) found that in vitro binding of ^{14}C-trichloroethylene to hepatic microsomes from male Sprague-Dawley rats was reduced in the presence of SKF-525A, an inhibitor of microsomal mixed-function oxidase, and increased either in the presence of 3,3,3-trichloropropene epoxide or with pretreatment of the animals with phenobarbital; consequently, these authors concluded that metabolic interaction between trichloroethylene and microsomes yields the epoxide as a probable contributor to the toxic properties of trichloroethylene. The mutagenicity study by Greim et al. (1975) described on page 50 herein was done to estimate the mutagenic activity of a series of chlorinated ethylenes, and the reported data indicate positive activity with compounds with an asymmetric chlorine arrangement (vinyl chloride, 1,1-dichloroethylene, trichloroethylene) and no activity with compounds having a symmetric chlorine arrangement (cis-1,2-dichloroethylene, tetrachloroethylene, trans-1,2-dichloroethylene). Results of the study by Greim et al. (1975) led Henschler and Bonse (1977) to postulate that trichloroethylene epoxide would have a rather high electrophilicity and potential for chemical reactivity with cellular macromolecules by virtue of its asymmetric chlorine arrangement. However, Henschler and Bonse (1978) subsequently hypothesized that trichloroethylene epoxide may be converted to trichloroacetaldehyde completely within the catalytic center of microsomal cytochrome P450, thus suggesting that this enzyme serves as a protective mechanism against possible toxic effects of the epoxide. To support their hypothesis, Henschler and Bonse (1978) concluded, from evidence described in their report, that carbon monoxide, a degradation product of trichloroethylene epoxide in aqueous solution, is not detected *in vivo* or in microsomal preparations after exposure to trichloroethylene which, according to the authors, would not be expected if the epoxide were reacting outside of the catalytic center. Nonetheless, there may be differences in the behavior of trichloroethylene epoxide in aqueous solution and biological tissue to make the relationship proposed by Henschler and Bonse (1978) unclear. In summary, there is experimental evidence that trichloroethylene is metabolically converted to an epoxide which can potentially produce toxic effects by chemically reacting with cellular macromolecules; however, the actual relationship of the epoxide as a metabolic intermediate, to the carcinogenic potential of trichloroethylene, remains undefined.

Hathway (1980) treated adult male pathogen-free Alderly Park mice with a single gavage dose of 2.0 ml 1,1,2-trichloroethylene/kg for identification of metabolites in urine and feces. The trichloroethylene sample was > 99.99% pure and was stabilized with 0.02% (w/v) triethylamine (no detectable epoxides) (personal communication with D. E. Hathway). A small amount of dichloroacetic acid was detected as a urinary metabolite, and it is indicated in the report that dichloroacetic acid has not yet been found as a product of trichloroethylene metabolism in other mammalian species. The authors postulated that, in conjunction with a rapid rate of epoxidation, large doses of trichloroethylene in mice produce saturation of enzymatic sites which catalyze the rearrangement of trichloroethylene oxide into chloral. An increase in chloral levels would occur relative to the rate-limiting lower activity of enzymes mediating chloral degradation. Consequently, an excess of trichloroethylene oxide would be produced which could be transformed into dichloroacetyl chloride, which could conceivably interact with DNA nucleosides to form cyclized products and dichloroacetic acid.

Numerous studies, including those described in Table 2, have been done to estimate the pulmonary absorption of trichloroethylene by humans. Depending on experimental conditions used, absorption percentages reported for studies described in Table 2 and below range from 35 to 76% with respect to exposure to 268 to 2,044 mg/m^3 air.

Monster et al. (1976) evaluated the absorption, excretion, and metabolism of trichloroethylene vapor in four men exposed to exposure levels of 376 and 753 mg/m^3 air (70 and 140 ppm) for 4 hours. The fraction of inhaled trichloroethylene retained in the body at rest was estimated to be 44.7% (43% to 49%) at 70 ppm and approximately 41.1% (37% to 48%) at 140 ppm. Mean estimated doses absorbed during the 4-hour exposure were 390 (320 to 470) and 755 (710 to 790) mg for the low and high exposure levels, respectively; hence, the absorbed dose appears to have been proportional to the amount inhaled. Of the total dose absorbed at the 70 ppm exposure level, estimated recovery included 10.5% (7% to 13%) of the dose as trichloroethylene in expired air, 42.8% (38% to 51%) of the dose as trichloroethanol in urine, and 21.0% (13% to 27%) of the dose as trichloroacetic acid in urine. Of the total dose absorbed at the 140 ppm exposure level, estimated recovery included 7.8% (7% to 9%) of the dose as trichloroethylene in expired air, 39.5% (31% to 45%) of the dose as trichloroethanol in urine, and 17.0% (8% to 25%) of the dose as trichloroacetic acid in urine. Blood levels of trichloroethanol and trichloracetic acid decreased with half-lives of 10 to 12 and 70 to 100 hours, respectively. The same volunteers were exposed to both doses in this study. Exposures to trichloroethylene were made in a 21.8 m^3 inhalation chamber with an air flow rate of 150 m^3/hour. Analytical measurements of atmospheric trichloroethylene levels were reported to be within ± 4% of the nominal concentrations. Analyses

TABLE 2. SUMMARY OF REPORTED PULMONARY ABSORPTION FOR TRICHLOROETHYLENE IN HUMANS
(adapted from the U. S. Environmental Protection Agency 1980)

Percent Absorption	Concentration (mg/m^3)	Duration of Exposure	Reference
75	---	30 minutes	Astrand and Gamberale 1978
53	540	30 minutes	Astrand 1975
50	1080	30 minutes	Astrand 1975
51-64	1043	5 hours	Bartonicek 1962
55	1355-2044	2.7 hours	Nomiyama and Nomiyama 1974
50	268	---	Ikeda 1977
56-60	540-1306	5 hours	Soucek et al. 1952
35-63	1076	7 hours	Stewart et al. 1970
58-70	440-850	5 hours	Soucek and Vlachova 1960

for trichloroethylene and its metabolites in expired air, blood, and urine were made for up to 9 days following exposure.

Trichloroethylene metabolism in five men, 28 to 34 years old, exposed to 72.3 ± 3 ppm (389 ± 16 mg/m^3 air) of its vapor 4 hours daily for 5 days was also studied by Monster et al. (1979). Estimated daily uptake ± S.D. of trichloroethylene was 6.6. ± 0.4 mg/kg lean body mass or 450 ± 51 mg. The fraction of inhaled trichloroethylene retained in the body was calculated to be 44% (35 to 49%); however, the authors stated that, taking into consideration physiological dead space and dead space in the gas mask worn by the volunteers during exposure with frequency of respiratory ventilation, an alveolar retention of about 70% can be calculated. At the end of each exposure, average trichloroethylene levels were 34 ug/l in expired air and 1.3 mg/l in blood; however, concentrations determined in blood and expired air 18 hours after each exposure were twice as high following the fifth exposure as compared to the first. Trichloroethylene levels in blood declined to approximately 0.2 mg/l by 100 minutes following each exposure. By the end of 5 treatment days, metabolite levels had increased as follows: trichloroethanol to 4.2 mg/l in blood and 217 mg/day in urine, and trichloroacetic acid to 31 mg/l in blood and 82 mg/day in urine. At 18 hours after each exposure, trichloroethanol levels in blood and expired air were 30% higher during the final 3 days of exposure compared to the first; thus, a diurnal pattern of excretion for this metabolite is evident. Conversely, trichloroacetic acid concentrations in blood increased both during and between exposures throughout the study. After 5 days of treatment, blood levels of trichloroethanol and trichloracetic acid decreased with half-lives of 8 to 10 hours and 70 to 90 hours, respectively. Of the total dose of trichloroethylene absorbed, 78% was recovered as unchanged trichloroethylene in expired air (11%), trichloroethanol in urine (43%), and trichloroacetic acid in urine (24%). Monster (1979) concluded that a relatively high and almost constant absorption of trichloroethylene vapor by humans occurs in conjunction with a high partition coefficient between blood and air ($\lambda_{b/g} = 15$) and a rapid metabolism (75%).

Fernandez et al. (1975) investigated patterns of absorption, excretion, and metabolism in three adult male human subjects at rest exposed to trichloroethylene vapor in air. The trichloroethylene sample was > 99.6% pure, but impurities were not identified. In one experiment the subjects were exposed to 97 ppm (530 mg/m^3) trichloroethylene for 8 hours. In a second experiment two of the subjects were exposed to 54 ppm (295 mg/m^3) trichloroethylene for 8 hours. Exposure concentrations were monitored by infra-red spectrophotometry and gas chromatography to within ± 4% of nominal levels. Pulmonary ventilation and respiratory rate were measured while the subjects were exposed in the inhalation chamber, and physiologic dead space and dead space in the equipment were considered in the calculation of alveolar ventilation. Trichloroethylene concentrations in alveolar air were estimated during exposure and for 70 hours thereafter. Levels of trichloroacetic acid and trichloroethanol were measured in urine collected during exposure and after exposure until the level of each substance was < 0.1 mg/l urine. Analyses of alveolar air and urine were done by gas chromatography techniques.

Results are described as mean ± S.D. Trichloroethylene concentrations in alveolar air were 12.9 ± 1.3 ppm and 26.1 ± 2.4 ppm during exposure to 54 and 97 ppm, respectively. Using an alveolar ventilation rate of approximately 6.0 l/min, the amount of trichloroethylene absorbed during exposure was calculated to be 554 ± 25 mg at 54 ppm and 1,069 ± 90 mg at 97 ppm. Hence, the absorbed dose was proportional to the amount inhaled. The fraction of inhaled trichloroethylene retained in the body was estimated to be 76.2 ± 2.5% at 54 ppm and 73.1 ± 2.5% at 97 ppm. Approximately 59% of the total dose was recovered in both experiments with 8.4 ± 1.1% as trichloroethylene in alveolar air, 17.7 ± 4.3% as trichloroacetic acid in urine, and 32.7 ± 3.6% as trichloroethanol in urine.

Differences in trichloroethylene metabolism between men and women were determined by Nomiyama and Nomiyama (1971). Five men and five women, 18 to 20 years old, were exposed to 250 to 380 ppm (1366 to 2077 mg/m^3 air) for 160 minutes, and amounts of exhaled trichloroethylene and urinary trichloroethylene metabolites were estimated. Respiratory uptake of trichloroethylene through alveolar membranes was approximately 54 to 58% of the inhaled amount with an average retention of 34 to 38% of the inhaled amount in both sexes. However, urinary excretion of trichloroacetic acid and trichloroethanol, respectively, was 32.6% and 48.6% in males and 43.9% and 42.7% in females of the retained trichloroethylene. In a subsequent study by Nomiyama and Nomiyama (1974), a respiratory uptake of about 55%, with retention of approximately 35%, of a trichloroethylene vapor concentration of 250 to 380 ppm (1366 to 2077 mg/m^3 air) inhaled for 2.7 hours by five men and five women was found; no sex difference in trichlorethylene absorption and retention was noted in this study.

Sato and Nakajima (1978) compared the metabolic fate of trichloroethylene in four men whose hands were immersed in liquid trichloroethylene for 30 minutes with that of men exposed to 100 ppm (550 mg/m^3 air) trichloroethylene vapor for 4 hours. Estimation of trichloroethylene levels in venous blood and both trichloroethylene metabolite concentrations in urine indicated that dermal uptake of trichloroethylene was approximately one-third of that via inhalation. However, for 2 hours post-exposure, tidal-air concentrations of trichloroethylene were about twofold greater in subjects dermally treated suggesting that a large amount of trichlorethylene absorbed by skin exposure is excreted, probably unchanged, through the lungs. Trichloroethylene concentrations in alveolar air of three human volunteers during and after immersion of the thumb in trichloroethylene liquid (98% pure) for 30 minutes were estimated by Stewart and Dodd (1964). The mean concentration of trichloroethylene in the breath reached a maximum of 0.5 ppm at approximately 15 minutes after treatment with a subsequent decline to 0.08 ppm by 2 hours. Excretion by other routes was not estimated.

Kimmerle and Eben (1973a) followed the metabolism of trichloroethylene in human subjects exposed in a 15 m^3 inhalation chamber. Technical grade trichloroethylene, once distilled, was used; however, the composition of the test sample was not described. Levels of trichloroethylene and/or its metabolites in blood and urine extracts and expired air were estimated by gas chromatography. Urinary trichloroacetic acid was assayed photometrically. In one experiment, two groups of four subjects each (four males and four females) were exposed to 44 ± 4 ppm (240 ± 22 mg/m^3 air) or 40 ± 7 ppm (219 ± 38 mg/m^3 air) trichloroethylene vapor for 4 hours. After exposure, trichloroethylene in the breath was monitored for 3.5 hours; blood levels of trichloroethylene, trichloroethanol, and chloral hydrate were measured for 96 hours; and urine was analyzed for trichloroethanol and trichloroacetic acid for up to 8 days. A repeated exposure experiment was also done in which three men and one woman were exposed to 48 ± 3 ppm (262 ± 16 mg/m^3 air) trichloroethylene vapor, 4 hours daily for 5 days. Analyses were done to detect trichloroethylene in expired air for 3.5 hours after the first exposure and 7 hours following the final treatment. Blood levels of trichloroethylene and trichloroethanol were measured before each exposure and for 7 or 8 hours after each exposure. Trichloroacetic acid and trichloroethanol amounts were determined in urine specimens collected for 24 hours following each exposure and during each day for 12 days after final exposure.

Data from the acute exposure experiment are presented as the range of values for the eight subjects. Levels of 0.02 to 0.48 ug trichloroethylene/ml blood and 0.71 to 1.78 ug trichloroethanol/ml blood were attained by the end of the 4-hour exposure. Following treatment, trichloroethylene was not detectable in blood by 4 hours, and blood levels of trichloroethanol declined to < 0.123 ug/ml by 96 hours. After the beginning of exposure, urinary trichloroacetic acid levels were 0.29 to 1.32 mg at 4 hours, 12.60 to 22.09 mg in females and 5.40 to 12.42 mg in males at 48 hours, and 5.15 to 7.80 mg at 120 hours; trichloroethanol levels in urine were 6.26 to 23.80 mg at 4 hours, 7.59 to 55.44 mg at 48 hours, and 0.86 to 3.65 mg at 120 hours (120-hour values were from three males and one female).

Results from the subacute exposure experiment are given as the data range for the four subjects. Blood levels of trichloroethylene were approximately 0.27 to 0.85 ug/ml at the end of each exposure and rapidly diminished to nondetectable levels by 5 hours following treatment. In contrast, trichloroethanol was still found at blood concentrations of 0.47 to 2.11 ug/ml at 20 hours after each exposure. Trichloroacetic acid levels in urine were 2.93 to 6.61 mg after the first exposure, increased to a maximum of 51.47 to 89.66 mg after the final exposure, and declined to 1.37 to 3.56 mg by 12 days post-exposure. Urinary trichloroethanol levels were 46.14 to 116.10 mg after initial treatment, increased to a maximum of 89.61 to 120.09 mg after the final exposure, and decreased to nondetectable to 0.20 mg by 12 days post-treatment.

In the acute and subacute exposure tests, 3.0 to 3.5 ppm trichloroethylene was detected in the breath at the end of each exposure with a reduction to 0.2 to 0.4 ppm by 3.5 hours post-treatment.

Kimmerle and Eben (1973a) were unable to detect chloral hydrate as a trichloroethylene metabolite in blood in their study on humans; however, Cole et al. (1975) found chloral hydrate as a trichloroethylene metabolite in man. Samples of venous blood were obtained from 15 patients at approximately 10 minutes after being anesthetized with trichloroethylene (dose level was not described) for analysis for chloral hydrate in plasma extracts by gas chromatography-mass fragmentography. Analysis of plasma extracts from the patients, plasma extracts to which chloral hydrate was added, and a standard solution of chloral hydrate revealed a similar peak-height ratio and the same retention time for each sample type. Chloral hydrate levels in plasma extracts were also monitored in a patient for 120 minutes following induction of anesthesia with 0.1% trichloroethylene for 10 minutes. A peak plasma level of 175 ng chloral hydrate/ml blood was attained by 30 minutes followed by a rapid decrease to 10 ng/ml by 90 minutes.

The detection of chloral hydrate, trichloroethanol, and trichloroacetic acid as trichloroethylene metabolites in humans by Kimmerle and Eben (1973a) and Cole et al. (1975) are in accordance with the metabolic scheme for trichloroethylene in Figure 1, page 10, and the detection of these metabolites in the metabolic study on trichloroethylene in rats by Kimmerle and Eben (1973b).

Trichloroethylene metabolism in male SPF-Wistar-II rats during and after acute and subacute inhalational exposures was investigated by Kimmerle and Eben (1973b). Technical grade trichloroethylene, once distilled, was used; however, the composition of the test sample was not described. Levels of trichloroethylene and/or its metabolites in tissue and urine extracts and expired air were measured by gas chromatography. Urinary trichloroacetic acid was assayed colorimetrically. Three groups of 20 rats each were exposed to 49, 175, or 330 ppm (270, 960, or 1,800 mg/m^3 air) trichloroethylene vapor for 4 hours to determine trichloroethanol and trichloroacetic acid levels in urine through 4 days following treatment in all animals and trichloroethylene concentrations in expired air during 8 hours post-exposure in three rats per group. In another experiment, five animals per treatment group were exposed to 54 or 3,160 ppm (300 or 17,268 mg/m^3 air) trichloroethylene vapor for 4 hours to estimate trichloroethylene, trichloroethanol, and chloral hydrate levels in blood for 60 minutes after treatment.

Another group of 20 rats was exposed to 55 ± 4 ppm (300 ± 20 mg/m^3 air) trichloroethylene vapor, 8 hours daily, 5 days/week, for 14 weeks. Total weekly urinary excretion of trichloroacetic acid and trichloroethanol from 10 animals was determined during the 14-week treatment period. Blood concentrations of trichloroethylene, trichloroethanol, and chloral hydrate in 10 animals were estimated during the second, third, fourth, sixth, ninth, and 14th weeks of treatment. At the end of treatment, hematologic analyses and liver and kidney function tests were done before sacrifice of the animals for necropsy examination, weighing of organs, and estimation of trichloroethylene residues in fat, brain, liver, kidney, heart, and spleen.

Comparable numbers of untreated controls which were similarly maintained with treatment animals were concurrently evaluated in the acute and subacute experiments.

With acute exposure, mean total urinary excretion of 342.5, 861.8, and 1029.1 ug trichloroacetic acid and 848.0, 2,285.2, and 4,066.4 ug trichloroethanol was found by 1 day post-treatment in low, mid, and high dose groups, respectively; only traces of these urinary metabolites were detected by 3 days in the low dose group and 4 days in the other groups. Trichloroethylene amounts in expired air were 51.1, 394.1, and 3,870.0 ug in low, mid, and high dose animals, respectively, at the end of exposure; however, these levels declined to nondetectable, 5.8 ug and 17.0 ug in the respective dose groups by 8 hours following treatment. Blood analyses revealed 0.8 ug trichloroethanol/ml blood and traces of chloral hydrate at 5 minutes following exposure to 54 ppm trichloroethylene, but by 60 minutes only 0.2 ug trichloroethanol/ml blood was found. After exposure to 3,160 ppm, 89.2 ug trichloroethylene, 1.74 ug trichloroethanol, and 1.43 ug chloral hydrate, each per ml blood, were detected at 5 minutes; by 60 minutes, 19.4 ug trichloroethylene, 1.75 ug trichloroethanol, and 1.06 ug chloral hydrate, each per ml blood, were found.

With subacute exposure, 1,069 ug trichloroethanol and 807 ug trichloroacetic acid were excreted in urine during week 1 of exposure. During week 14 of treatment, urinary excretion of metabolites increased to 2,692 ug trichloroethanol and 1,279 ug trichloroacetic acid. Trichloroethylene was not found in blood, and the data range for weekly analyses indicated that blood levels of trichloroethanol (0.68 to 1.01 ug/ml blood) and chloral hydrate (0.11 to 0.45 ug/ml blood) were basically constant during the course of treatment. Trichloroethylene was not found in body tissues, and hematology, liver, and renal tests were stated to be normal. Although liver weights and liver/body weight ratios were reported to be significantly increased ($P < 0.01$) in treatment animals compared to controls, the data are not presented in the publication.

The detection of chloral hydrate, trichloroethanol, and trichloroacetic acid in rats by Kimmerle and Eben (1973b) are in accordance with the metabolic scheme for trichloroethylene presented in Figure 1, page 10, and with the determination of these metabolites in the metabolic studies of trichloroethylene in humans by Kimmerle and Eben (1973a) and Cole et al. (1975).

Results of a metabolism study on trichloroethylene in mice and rats have been reported (Stott et al. 1981). Trichloroethylene (> 99.9% pure, amine stabilized) and ^{14}C-trichloroethylene (> 99% pure, specific activity

= 11.3 mCi/mmol were used. Test animals were male B6C3F1 mice (17 to 26 g) and male Osborne-Mendel rats (190 to 250 g). Mice were housed in groups, and rats were caged individually.

Sixteen animals per exposure level were exposed to 10 or 600 ppm ^{14}C-trichloroethylene for 6 hours in a 30 l glass inhalation chamber at an airflow of 5 l/min (mice) or 10 l/min (rats). For the metabolism study, four animals in each exposure group were acclimated for at least 1 day in metabolic cages before treatment. Chamber concentrations, monitored spectrophotometrically, were within ± 10% of nominal concentrations. Exposure levels of ^{14}C-trichloroethylene were measured by liquid scintillation counting. Mice were exposed to specific activities of 665 uCi/mmol trichloroethylene at 10 ppm and 15.0 uCi/mmol at 600 ppm, and rats were exposed to 174 uCi/mmol at 10 ppm and 4.5 uCi/mmol at 600 ppm.

Excretion of ^{14}C activity in urine, feces, and expired air from the four animals per exposure group in the metabolism study was followed until the animals were decapitated at 50 hours following treatment. Micromole equivalents of ^{14}C-trichloroethylene were determined by liquid scintillation counting in urine, trapped expired CO_2, toluene-desorbed trapped expired ^{14}C-trichloroethylene, and oxidized samples of liver, kidney, skin, feces homogenate, and carcass homogenate.

To estimate macromolecular binding, the remaining 12 animals per exposure group were divided into groups of four for sacrifice at 0, 6, and 24 hours post-exposure. Protein content in trichloroacetic acid-precipitable material and ^{14}C activity were estimated in homogenized samples of liver and kidney from each treated animal.

Pathology and DNA synthesis in liver and kidney from mice and rats dosed with trichloroethylene in corn oil by gavage were investigated. In each experiment, control groups of rats and mice were given similar amounts of corn oil alone. In one experiment, daily doses of trichloroethylene were given to mice (2,400 mg/kg/day) and rats (1,100 mg/kg/day) for 3 days; 3 to 4 hours after each dose, subcutaneous injections of ^{3}H-thymidine at levels of 1 mCi (15.2 ug, 10 ml/kg) in mice and 0.1 mCi(1.52 ug, 1 ml/kg) in rats were made. In a second experiment, rats and mice were given the above trichloroethylene doses 5 days per week for 3 weeks; ^{3}H-thymidine (0.1 mCi, 6.42 ug, 10 ml/kg) was injected into mice during the final 5 days of treatment and was administered into rats "... via implantation between the shoulder blades of an osmotic pump ... containing 0.1 mCi and 1 ug ^{3}HdT on day 15 of the experiment." A dose-response study was also done in which mice were given 250, 500, 1,200, or 2,400 mg/kg/day of trichloroethylene 5 days per week for 3 weeks with injection of ^{3}H-thymidine, done as indicated for the 3-day study, during the final 4 days before sacrifice.

At the end of each of the above experiments with gavage dosing, animals were sacrificed for removal of livers and kidneys. Extraction and quantitation of DNA was done, and ^{3}H-thymidine incorporation, indicative of DNA synthesis, was estimated by liquid scintillation counting. Liver and kidney specimens were examined microscopically.

Alkylation of DNA _in vivo_ was evaluated in DNA from livers of mice killed 5 hours after gavage administration of 1,200 mg/kg of ^{14}C-trichloroethylene (> 500 uCi/mouse). Isolation of DNA was done by a technique based on the purification methods of Marmur (1961) and Kirby (1957), and DNA content and ^{14}C activity were measured. Half of each DNA sample was enzymatically degraded to nucleosides, and the remainder was digested in 0.1 M HCL to separate purine bases and phosphate sugars with minimal loss of alkylated products. The DNA digests were analyzed by high pressure liquid chromatography.

Data were statistically analyzed by the Dunnett's or Student's t-test.

Results of the metabolism study on mice and rats exposed to trichloroethylene by inhalation are described in Tables 3 and 4. At both exposure levels (10 and 600 ppm), metabolized trichloroethylene in mice was estimated to be 98 to 99% of the total body burden. In rats, metabolized trichloroethylene comprised approximately 98 and 79% of the total body burden at 10 and 600 ppm, respectively. Rats eliminated proportionately more ^{14}C-trichloroethylene in expired air at the high dose than at the low dose compared to mice. Estimated trichloroethylene metabolism, on a body weight basis, was 2.2-fold and 3.6-fold greater in mice compared to rats at the low and high dose, respectively. It should be noted that the authors stated, without presenting their data, that B6C3F1 mice dosed orally with 790 mg/kg of trichloroethylene metabolized 75% of the body burden and eliminated the remaining 25% as unmetabolized trichloroethylene in the breath.

Macromolecular binding of ^{14}C-trichloroethylene metabolite(s) in liver and kidney from mice and rats exposed to 10 and 600 ppm trichloroethylene is evident from data in Table 5. Binding at 600 ppm was considerably greater in liver and kidney from mice, whereas binding at 10 ppm was similar between species. The results provide evidence for a larger metabolic capacity in B63CF1 mice to produce biologically reactive metabolites.

Mice given 2,400 mg/kg/day of trichloroethylene by gavage for 3 days had increased liver/body weights (120%), reduced hepatic DNA content/g tissue (90% of control), and increased hepatic DNA synthesis (222% of control); however, liver weights, hepatic DNA content, and hepatic DNA synthesis between control rats and rats given 1,100 mg/kg/day of trichloroethylene for 3 days were similar.

Measurements of organ/body weights, DNA content, and DNA synthesis in liver and kidney from mice and rats dosed by gavage with 2,400 and 1,100 mg/kg/day of

TABLE 3. RECOVERY OF RADIOACTIVITY FOR 50 HOURS POST INHALATION EXPOSURE OF MALE B6C3F1 MICE TO 10 PPM OR 600 ppm 1,1,2-TRICHLOROETHYLENE-UL-^{14}C FOR 6 HR*

(Stott et al. 1981)

	10 ppm		600 ppm	
	mole eq TRI/ kg body wt	%†	mole eq TRI/ kg body wt	%†
Expired				
1,1,2-TRI	0.617 (0.182)	0.79	76.2 (27.3)	2.4
CO_2	7.38 (0.383)	9.4	299 (37.5)	9.5
Urine	58.1 (18.8)	74.0	2278 (505)	72.6
Feces	2.92 (0.542)	3.7	115 (24.6)	3.7
Cage Wash§	1.10 (0.446)	1.4	85.5 (65.5)	2.7
Skin	2.33 (1.13)	3.0	56.3 (11.9)	1.8
Liver	2.40 (0.401)	3.1	72.2 (10.4)	2.3
Kidney	0.310 (0.310)	0.39	11.1 (3.18)	0.35
Carcass	1.50 (0.316)	1.9	146 (57.9)	4.6
Total Body Burden	78.5 (17.9)		3138 (616)	
Total Metabolized	77.9 (18.1)	99.2	3062 (592)	97.6

---

*Average of four animals (± Standard Deviation).

†Percentage of recovered radioactivity.

§Primarily due to urine.

TABLE 4. RECOVERY OF RADIOACTIVITY FOR 50 HOURS POST INHALATION EXPOSURE OF MALE OSBORNE-MENDEL RATS TO 10 PPM OR 600 PPM 1,1,2-TRICHLOROETHYLENE-UL-^{14}C FOR 6 HR*
(Stott et al. 1981)

	10 ppm			600 ppm		
	mole eq TRI/ kg body wt		%†	mole eq TRI/ kg body wt		%†
Expired						
1,1,2-TRI	0.778	(0.103)	2.1	227	(6.40)	21.1
CO_2	1.71	(0.295)	4.8	31.2	(2.10)	2.9
Urine	22.6	(5.96)	63.1	594	(79.8)	55.3
Feces	2.52	(0.253)	7.0	38.9	(3.47)	3.6
Cage Wash§	0.537	(0.204)	1.0	15.1	(10.7)	1.4
Skin	3.78	(1.21)	10.6	76.2	(30.0)	7.1
Liver	1.01	(0.085)	2.8	16.3	(1.13)	1.6
Kidney	0.131	(0.009)	0.37	1.72	(0.083)	0.16
Carcass	2.82	(0.898)	7.9	75.1	(27.7)	7.0
Total Body Burden	35.8	(5.64)		1075	(87.2)	
Total Metabolized	53.0	(5.64)	97.8	847	(81.6)	78.9

---

*Average of four animals (± Standard Deviation).

†Percentage of recovered radioactivity.

§Primarily due to urine.

TABLE 5. HEPATIC AND RENAL MACROMOLECULAR BINDING OF 1,1,2-TRICHLOROETHYLENE-UL-^{14}C METABOLITE IN MALE B6C3F1 MICE AND OSBORNE-MENDEL RATS EXPOSED TO 10 PPM OR 600 PPM FOR 6 HOURS*

(Stott et al. 1981)

Exposure	Tissue	Test Animal	Binding (pmoles eq ^{14}C-TRI/ g protein)	Ratio (Mouse/Rat)
10 ppm	Liver	Mouse	0.318 (0.004)	1.2†
		Rat	0.286 (0.013)	
	Kidney	Mouse	0.168 (0.014)	1.1
		Rat	0.153 (0.007)	
600 ppm	Liver	Mouse	20.4 (2.49)	4.3†
		Rat	4.72 (0.415)	
	Kidney	Mouse	5.06 (0.667)	3.0†
		Rat	1.77 (0.200)	

---

*Avg. of four animals (± Standard Deviation) at time of maximum binding post exposure (liver = 0 hr., kidney = 3 hr).

†P < 0.01.

trichloroethylene, respectively, are shown in Table 6. Similar measurements in liver alone obtained from the 3-week dose-response study in mice are described in Table 7. Increases in organ/body weight and DNA synthesis were evident in rat and mouse liver, and DNA content/ug tissue was reduced in mouse liver. Rat and mouse kidneys were unaffected by trichloroethylene treatment.

Histopathologic findings on mouse liver in the dose-response study progressed from increased eosinophilic staining of hepatocytes at 250 and 500 mg/kg/day to centrilobular hepatocyte swelling at 1,200 mg/kg/day to more severe centrilobular hepatocyte swelling, giant cell inflammation, and mineralized cells at 2,400 mg/kg/day. Liver from mice treated for 3 days showed general centrilobular hepatocyte swelling, some necrosis, and increased mitotic activity. Histopathologic findings in rat liver and rat and mouse kidney were reported as unremarkable.

In the DNA alkylation experiment, an average DNA alkylation value of 0.62 ± 0.42 alkylations/10^6 nucleotides was estimated in liver from three mice given a single dose of 1,200 mg/kg of ^{14}C-trichloroethylene by gavage. No DNA alkylation was detected in liver from a fourth mouse. Carbon-14 activity was associated with purine bases. Although the DNA isolate was purified by the chromatographic method used, the authors indicated that the alkylation values obtained should be considered maximal since there could have been ^{14}C contamination from other sources, retention times of the peaks were shorter than those for most alkylated bases, and possible adducts were not identified. In comparison with the amount of DNA alkylation obtained with trichloroethylene treatment, the investigators cite evidence by Lawley (1976) in which mice incur hundreds of alkylations when treated with potent genotoxins such as dimethylnitrosamine and methylnitrosourea; however, a positive control agent was not evaluated in their study. Furthermore, alkylation of DNA in negative

TABLE 6. ORGAN TO BODY WEIGHT RATIOS, DNA CONTENT, DNA SYNTHESIS, AND HISTOPATHOLOGY DATA FROM MALE B6C3F1 MICE AND MALE OSBORNE-MENDEL RATS ADMINISTERED 1,1,2-TRICHLOROETHYLENE BY GAVAGE FOR 3 WEEKS (5 days/wk)*
(adapted from Stott et al. 1981)

Experiment	Dose Level	% Organ Wt / Body Wt	ug DNA/ g Tissue	DNA Synthesis	
				DPM ^{3}HdT / ug DNA	Treated / Control
			Rats		
Control	(corn oil)				
	Liver	3.77 (0.21)	3268 (396)	1.21 (0.61)	
	Kidney	0.79 (0.04)	3646 (805)	5.50 (2.34)	
Treated	(110 mg/kg)				
	Liver	4.43 (0.21)†	3272 (262)	2.12 (0.96)†	1.75
	Kidney	0.82 (0.04)	3530 (59.4)	6.31 (1.75)	1.15
			Mice		
Control	(corn oil)				
	Liver	5.28 (0.25)	2992 (210)	11.06 (2.25)	
	Kidney	1.49 (0.14)	5626 (640)	10.35 (2.40)	
Treated	(2400 mg/kg)				
	Liver	8.95 (0.72)†	2267 (125)†	20.02 (6.96)†	1.81
	Kidney	1.49 (0.11)	4979 (600)	8.39 (1.11)	0.81

*Average of 10 to 12 animals (± Standard Deviation).
†P < 0.01.

TABLE 7. LIVER TO BODY WEIGHT RATIOS, DNA CONTENT, DNA SYNTHESIS, AND HISTOPATHOLOGY DATA FROM MALE B6C3F1 MICE ADMINISTERED 1,1,2-TRICHLOROETHYLENE BY GAVAGE FOR 3 WEEKS (5 days/wk)*
(Stott et al. 1981)

			DNA Synthesis	
Dose Level	% Liver Wt Body Wt	ug DNA g Hepatic Tissue	dpm^{3}HdT ug DNA	Treated Control
Control (corn oil)	4.85 (0.28)	2722 (203)	128 (27.5)	
Treated				
250 mg/kg	5.20 (0.53)	2517 (220)	108 (34.0)	0.84
500 mg/kg	5.46 (0.61)†	2391 (203)†	87.6 (15.4)§	0.68
1200 mg/kg	6.56 (0.42)†	2143 (122)†	140 (41.0)	1.09
2400 mg/kg	8.57 (0.46)†	2046 (356)†	156 (52.6)	1.22

*Average of 10 to 12 animals (± Standard Deviation).
†$P < 0.01$.
§$P < 0.05$.

control mice was not done for comparison with treated mice.

The results of the metabolism study by Stott et al. (1981) provide evidence for a greater ability of male B6C3F1 mice to metabolize trichloroethylene to reactive products compared to male Osborne-Mendel rats. Increased proportional exhalation of unchanged trichloroethylene from rats exposed to 600 ppm trichloroethylene indicates saturability of metabolic processes at this exposure level. Although the amount of recovered ^{14}C activity, excluding expired ^{14}C-trichloroethylene, shown in Tables 3 and 4 was interpreted as the amount metabolized, a more precise estimate of metabolism possibly could have been obtained by an attempt to identify and quantify metabolites in the samples analyzed. Additionally, the extent to which the higher specific activity of ^{14}C in the mouse exposure concentrations possibly could have contributed to the increased body burdens of ^{14}C in mice is not certain.

In the experiments with gavage dosing, the greater potential for liver toxicity in mice compared to rats was demonstrated by increased DNA synthesis and tissue lesions found in the former species. The lack of similar effects of trichloroethylene on kidney in both species indicates that the liver in male B6C3F1 mice has the greatest sensitivity to trichloroethylene toxicity of the two organs (liver and kidney) examined in the gavage dosing experiments. There were differences in procedures for administering ^{3}H-thymidine (^{3}HdT) between species and among experiments, and these differences might have possibly had some influence on the DNA synthesis results. For example, liver from control mice in the 3-week study described in Table 6 contained 11.06 dpm ^{3}HdT/ug DNA after treatment with 0.1 mCi ^{3}HdT, and liver from control mice in the 3-week dose-response study described in Table 7 contained 128 dpm ^{3}HdT/ug DNA after treatment with 1.0 mCi ^{3}HdT. Nonetheless, differences between responses, including pathology, of rats and mice after gavage treatment are apparent.

Results of the DNA alkylation experiment by Stott et al. (1981) and the overall mutagenicity data base as discussed in the mutagenicity section of this document furnish evidence that trichloroethylene has genotoxic potential in the liver, albeit low. The authors concluded that, considering the demonstrated weak genotoxicity (mutagenicity) of trichloroethylene and the hepatotoxicity in B6C3F1 mice given trichloroethylene orally at a dose that produced liver carcinomas in mice in the NCI (1976) bioassay further discussed herein, induction of liver carcinomas by trichloroethylene in this mouse strain is due to an epigenetic mechanism resulting from repeated cytotoxicity or enhancement of pre-existing hepatic oncogenicity factors. Induction of liver carcinomas from treatment of B6C3F1 mice with trichloroethylene by an epigenetic mechanism is possible; however, the etiology for a specific epigenetic mechanism remains to be defined.

Forssman and Holmquist (1953) conducted two series of experiments, one in which rats were exposed to 59 to 82 mg trichloroethylene vapor/l air (10,727 to 14,909 ppm) for 36 to 37 minutes and the other in which rats were exposed to 73 to 86 mg trichloroethylene vapor/l air (13,272 to 15,636 ppm) for 60 minutes. Of the inhaled quantity, 21 to 28% and 32 to 69% was exhaled with shorter and longer exposure, respectively. The amount of retained trichloroethylene determined to be excreted as trichloroacetic acid in urine was 1.2 to 3.9% and 3.2 to 7.8% with shorter and longer exposure, respectively; the authors concluded that the rest of the retained trichloroethylene was eliminated primarily as unidentified conversion products.

In a study by Daniel (1963), the following excretory pattern emerged on administration of single doses of 4.0 to 8.6 uCi (40 to 60 mg/kg) of ^{36}Cl-trichloroethylene to Wistar rats by gavage. Of 100% of the dose, 10 to 20% was eliminated in the urine with 1 to 5% as trichloroacetic acid and 10 to

15% as trichloroethanol, 0 to 0.5% was excreted unchanged in the feces, and 72 to 85% was removed unchanged via the lung to yield a total excretion level of 92 to 96%. The half-life of expiration of trichloroethylene from the lung was 5 hours, and radioactivity was detected in urine for approximately 18 days. Specific activities of the trichloroacetic acid and trichloroethanol metabolites and the administered trichloroethylene were about equal to suggest an intramolecular rearrangement of trichloroethylene with no exchange of chloride with the body chloride pool.

Trichloroacetic acid and trichloroethanol have also been found to be urinary trichloroethylene metabolites in dogs exposed to trichloroethylene vapor (Barrett and Johnston 1939, Butler 1949).

Barrett et al. (1939) found trichloroethylene residue levels in fat, which contained the most residue of the tissues analyzed, skeletal muscle, liver, blood, kidney, lung, and heart from two dogs and two rabbits anesthetized with trichloroethylene; however, although an exposure period of 25 to 28 minutes is mentioned, the exposure conditions for the animals are not clearly described in the study report. The colorimetric Fujiwara method was used to detect trichloroethylene in this study.

Laham (1970) reported that trichloroethylene passes through the human placental barrier _in vivo_. Ten pregnant women were placed under anesthesia with a mixture of trichloroethylene, nitrous oxide, and oxygen. Immediately upon birth of the children, blood was taken from the umbilical vein and the forearm vein of the mother, and the presence of trichloroethylene was determined colorimetrically. Helliwell and Hutton (1950) found trichloroethylene in the fetal blood of pregnant sheep and goats within 5 and 10 minutes of exposure (earliest sampling times) respectively, and trichloroethylene vapor concentrations of 0.532 to 1.51 volume percent. Trichloroethylene was detected in blood samples from the maternal carotid artery and the umbilical vein.

IV. MUTAGENICITY AND CELL TRANSFORMATION*

A number of studies employing various assays have been conducted to assess the mutagenicity of trichloroethylene. As indicated in Table 8, both positive and negative results have been obtained depending on the test system used, and, as described in the test, conditions of test. In several of these studies, the composition of the trichloroethylene sample used was not described.

TESTING OF TECHNICAL GRADE MATERIAL

Because of the possibility that positive results could have been due to mutagenic contaminants in the trichloroethylene samples, two groups of investigators, Henschler et al. (1977) and Loprieno et al. (1979), chemically analyzed samples of trichloroethylene and subsequently tested them for mutagenicity. Henschler et al. (1977) determined that a sample of technical grade trichloroethylene used in the National Cancer Institute (NCI 1976) carcinogenicity bioassay contained the following impurities (% w/w) by gas chromatography-mass spectrometry analysis: epichlorohydrin, 0.22; 1,2-epoxybutane, 0.20; carbon tetrachloride, 0.05; chloroform, 0.01; 1,1,1-trichloroethane, 0.035; diisobutylene, 0.020; ethylacetate, 0.052; pentanol-2, 0.015; butanol, 0.051. In *Salmonella typhimurium* strain TA 100, plus S-9 mix from PCB-induced rat livers, mutagenic activity was strong with epichlorohydrin and 1,2-epoxybutane when tested individually. (Although they are direct-acting mutagens, these substances were not tested without activation.) The mutation frequency for these two compounds (extrapolated from Figure 1 of the paper) was > 2000 revertants at 12.8 mM and 750 revertants at 506.9 mM, respectively, but no increased mutation frequency was obtained with the purified grade of trichloroethylene. The mutation frequency for the

---

*Prepared by the Reproductive Effects Assessment Group.

TABLE 8. RESULTS OF SHORT-TERM MUTAGENICITY STUDIES
(adapted from Loprieno et al. 1979)

Organism	Technique	Purity	Results*	Reference
1. *Salmonella typhimurium*	*in vitro*	Purified	-	Henschler et al. 1977
		Technical	+	Margard 1978
		Purified	-	Margard 1978
		Purified	-	Waskell 1978
		Purified†	+	Simmon et al. 1977
		Purified†	+	Baden et al. 1979
		Purified†	+	Bartsch et al. 1979
2. *Escherichia coli*	*in vitro*	Purified	+	Greim et al. 1975
3. *Schizosaccharomyces pombe*	h.m.a. (mice)§	Technical	-	Loprieno et al. 1979
		Purified	-	Loprieno et al. 1979
		Purified	-	Mondino 1979
4. *Saccharomyces cerevisiae*	*in vitro*	Purified	+	Callen et al. 1980
		Not reported	+	Shahin and Van Borstel 1977
		purified	+	Bronzetti et al. 1978
	h.m.a. (mice)§	Purified	+	Bronzetti et al. 1978
5. *Drosophila melanogaster*	*in vivo*	Technical	-	Abrahamson and Valencia 1980
6. Mouse (somatic mutation)	*in vivo*	Purified	+	Fahrig 1977
(dominant lethal)	*in vivo*	Purified	-	Slacik-Erben et al. 1980
(SCE)	*in vivo*	Anesthetic Grade	-	White et al. 1979
7. *Tradescantia* (somatic mutation)	*in vivo*	Not reported	+	Schairer et al. 1978
8. Human (hypodiploid cells)	*in vivo*	Technical	+	Konietzko et al. 1978

---

* + = positive results; - = negative results. See discussion of each report in body of the text for doses employed, responses observed, and discussion of strengths and limitations of the results.

† = equivocal < twofold increase.

§h.m.a. = host-mediated assay.

technical grade trichloroethylene extrapolated from Figure 1 was roughly 140 revertants at each dose both with and without metabolic activation; the spontaneous revertant count for histidine-independent colonies was 138 ± 8 (without activation) and 242 ± 10 (with activation) colonies per plate. Test samples were added directly to the top agar; no precautions were reported to have been taken to prevent excessive evaporation of the test compound and thereby ensure exposure of the test organisms. Thus, the significance of the negative response obtained with the technical grade sample of trichloroethylene is weakened by the possibility that the material may have evaporated before adequately exposing the indicator cells. The cells were incubated for 48 hours at 37°C before colonies were counted, and each assay was done in triplicate. No data are presented concerning toxicity caused by the tested substances. Carbon tetrachloride, chloroform, and 1,1,1-trichloroethane were reported to be inactive both with and without metabolic activation in this system, but no data are presented to support this claim. Based on the results, these authors concluded that carcinogenic activity of the technical grade trichloroethylene in the NCI bioassay experiment was probably predominantly if not exclusively due to the epoxide contaminants.

Employing the yeast Schizosaccharomyces pombe in a host-mediated assay with B6C3F1 mice, Loprieno et al. (1979) reported findings similar to those obtained in bacteria by Henschler et al. (1977). In this study the mutagenic potential of technical grade trichloroethylene (composition, % (w/w): trichloroethylene, 99; 1,2-epoxybutane, 0.19; epichlorohydrin, 0.09; ethylacetate, 0.04; n-methyl pyrrole, 0.02; diisobutylene, 0.03) was compared with that of a purified grade of trichloroethylene (composition, trichloroethylene, 99.98% (w/w): chloroform, 10 ppm; carbon tetrachloride, 17 ppm; 1,1,2-trichloroethane, 21 ppm; 1,1,2,2-tetrachloroethane, 14 ppm; other contaminants, 16 ppm). One and one-half ml (2×10^9 cells/ml) of yeast cell suspension (SP ade 6-60/rad 10-198h$^-$) was injected into the peritoneal cavity of mice untreated (controls) or treated with 0.5 ml trichloroethylene administered by gavage, and cells were removed from the peritoneum and plated for counting 3, 6, or 16 hours after treatment. Each assay was performed in at least three animals. The report states that results of this test (summarized in Table 9) indicate that technical grade trichloroethylene was weakly positive, but the linear regression analysis presented in the report was performed incorrectly and the correlation coefficient was reported to be statistically significant ($r = 0.86$, $df = 13$). The true r value (0.35) is not statistically significant. Similarly, the same dose of purified grade trichloroethylene produced no increase in mutation frequency. The mutagenic response to epichlorohydrin tested by itself was, however, positive ($3.71 \pm 1.05 \times 10^{-4}$ mutants at 50 mg/kg) as was the response of the positive control dimethylnitrosamine ($16.73 \pm 3.24 \times 10^{-4}$ mutants at 100 mg/kg) indicating that for these chemicals the assay was working properly. The number of mutants in the controls for the technical grade product was much less, by 44% and 35%, respectively, than those in the controls for the purified grade trichloroethylene and epichlorohydrin. No data are presented bearing on the ability of the administered trichloroethylene samples to reach the indicator organisms (e.g., chemical binding). The negative responses obtained for the pure and technical trichloroethylene samples tested in this study may indicate that the material is not mutagenic, but it may also be that although trichloroethylene (or an active metabolite) is mutagenic, it did not reach the indicator organisms under the test conditions employed.

Mondino (1979) also reported negative results for purified grade trichloroethylene in a yeast host-mediated assay. He used Schizosaccharomyces

TABLE 9. MUTAGENICITY OF TRICHLOROETHYLENE AND EPICHLOROHYDRIN IN *S. POMBE* BY MEANS OF THE HOST-MEDIATED ASSAY IN B6C3F1 MICE

(16 hours of incubation)
(Loprieno et al. 1979)

Treatment	Dose mg/kg	Mutants M.F. x 10^{-4} (M ± S.E.)
Trichloroethylene technical grade	None (control)	0.94 ± 0.08*
	500	1.33 ± 0.28
	1,000	1.32 ± 0.29
	2,000	1.61 ± 0.45
Methyl methanesulfonate	50	4.16 ± 0.54
Trichloroethylene purified grade	None (control)	1.45 ± 0.06
	1,000	1.59 ± 0.22
	2,000	1.36 ± 0.21
Dimethylnitrosamine	100	16.73 ± 3.24
Epichlorohydrin	None	1.66 ± 0.03
	2	1.50 ± 1.20
	10	2.05 ± 0.51
	50	3.71 ± 1.05

*Lower than the other negative control values.

pombe and adult male B6C3F1 mice. The ingredients in the trichloroethylene sample were identified as follows: trichloroethylene, 99.9%; water, evaporation residue, chloroform, 1,2-dichloroethane, carbon tetrachloride, 1,1,2-trichloroethane, other impurities, all ≤ 20 ppm; diisopropylamine, 198 ppm; diisobutylene, 104 ppm; butylhydroxytoluene, 12 ppm. Yeast cell suspension was injected into the peritoneum of the mice, and treated mice received 500, 1000, or 2000 mg/kg dosages of trichloroethylene by gavage. Three animals comprised each treatment group as well as the untreated control and positive control (100 mg/kg of dimethylnitrosamine) groups. After a 16-hour incubation period the animals were sacrificed for removal of yeast cells from the peritoneum for plating and counting. Plates were incubated at 32°C for 4 to 5 days prior to mutant colony counting. Trichloroethylene was not detected as mutagenic in this study. Mean numbers (± S.D.) x 10^{-4} of mutant (white as opposed to normal red) colonies were as follows: control, 1.80 ± 0.48; low dose, 1.18 ± 1.18; mid-dose, 1.03 ± 0.91; high-dose, 1.84 ± 0.28; positive control 31.1 ± 3.31. As was the case for the study by Loprieno et al. (1979), the negative result obtained by Mondino may be due to the fact that trichloroethylene is not mutagenic, or it may be due to the fact that although trichloroethylene is mutagenic, the compound or an active metabolite did not reach the DNA of the test organisms or it did not reach it in sufficient concentration to yield a detectable positive response. Furthermore, it should be noted that the summed spontaneous mutation frequency in the forward mutation assay for all the various loci involved in the synthesis of adenine in Schizosaccharomyces pombe is very high, 1 x 10^{-4}, and weak mutagens may not yield a statistically significant response in this system. An improvement in the host-mediated assays conducted by Loprieno et al. (1979) and Mondino (1979) would have been the use of intrasanguineous injection of yeast to evaluate its mutagenic potential with respect to specific organ sites, such as was done in the host-mediated assay by Bronzetti et al. (1978) described on pages 54 to 58. This method of testing provides an opportunity for recovery of the indicator of organisms from different organs of the host, and thus, makes it possible to detect mutagenesis caused by metabolites which are tissue specific.

The mutagenic properties of technical grade trichloroethylene in the XV 185-14C haploid strain of *Saccharomyces cerevisiae* were assessed *in vitro* by Shahin and Von Borstel (1977). With respect to the genotype of this strain, *ade 2-1*, *arg 4-17*, *lys 1-1*, and *trp 5-48* are suppressible mutants of the ochre variety; *his 1-7* can be reverted by true back mutation or second site base substitutions; and *hom 3-10* is considered a frameshift mutant. The ability of trichloroethylene to induce mutations at the lys 1-1, his 1-7, and hom 3-10 loci was assessed in this study. Stock cultures were incubated in liquid YPG medium at 30°C until a stationary growth phase was achieved. Cells were harvested, washed, and adjusted to a population of 1×10^8 cells/ml. In order to estimate the mutagenic effect of trichloroethylene in the absence of metabolic activation, cells treated with 1.11, 5.56, 111.1 or 222.2 mM of trichloroethylene during incubation at 30°C were withdrawn at 2, 6, 24, and 48 hours for plating and counting of colonies. Trichloroethylene mutagenicity was also evaluated by incubating cells for 1 or 4 hours at 37°C with 1.11, 5.56, 111.1, or 222.2 mM of trichloroethylene in the presence of a metabolic activation system derived from male mouse liver homogenate (uninduced) for subsequent plating and counting of colonies. No precautions were reported to have been taken to prevent excessive evaporation of the compound and ensure exposure to the test organism, but the reported toxicity indicates this was not a problem and that the cells were probably exposed. Treated cells were compared with similarly maintained untreated control and positive control (222.2 mM of

2-acetylaminofluorene without metabolic activation and 8 ug/ml of ethyl methanesulfonate with metabolic activation) cells in these experiments. Plates were maintained at 30°C for 5 days to estimate survival. Assays were performed in triplicate.

In the study described above, trichloroethylene at concentrations of 111.1 mM and 222.2 mM in the presence of metabolic activation increased the reversion frequencies at the lys 1-1, his 1-7, and hom 3-10 loci (Table 10). These trichloroethylene levels were also highly toxic to the cells both with and without metabolic activation based on measurements of survival (> 99% cell killing), but increased incidences of mutation were observed at three independent loci in the tests conducted with metabolic activation. The positive control in these experiments (EMS) was inappropriate because it does not require metabolic activation for its activity. The metabolic activation system employed may not have been working efficiently, but the positive result obtained with activation, compared to the test conducted without activation, suggests that some *in vitro* metabolism did occur. Trichloroethylene was ineffective as a mutagen without metabolic activation. For the maximum 48-hour treatment without activation, the number of revertants per 10^7 survivors for the three loci individually was 10 to 15 for untreated controls (98% survival), 12.8 to 21.4 for 1.11 mM (73% survival), 11.4 to 17.1 for 5.56 mM (64% survival), and 0 for 111.1 mM and 222.2 mM (< 0.1% survival) of trichloroethylene. The lack of revertants with the two highest trichloroethylene concentrations appears to correspond to failure of the cells to survive after plating. The largest number of revertants per 10^7 survivors produced by 2-acetylaminofluorene (222.2 mM) was 102 (lys 1-1) after a 6-hour treatment, 673 (his 1-7) and 488 (hom 3-10) after 24-hour treatment. The concentration effect for trichloroethylene mutagenicity is not clear in this study since levels between 5.56 mM

TABLE 10. REVERSION FREQUENCIES OF SACCHAROMYCES CEREVISIAE STRAIN XV185-14C AFTER TREATMENT WITH VARIOUS COMPOUNDS IN THE PRESENCE OF METABOLIC ACTIVATION
(adapted from Shahin and Von Borstel 1977)

Compounds	Treatment (hours)	Revertants per 10^7 survivors			Survival (%)
		lys 1-1*	his 1-7*	hom 3-10*	
Controls (no metabolic activation)	1	15.3 (132)	19.5 (168)	8.6 (74)	100
	4	19 (139)	20 (147)	14.9 (109)	85
Controls (metabolic activation)	1	15.3 (110)	18.5 (134)	9.4 (68)	84
	4	13.2 (112)	19.2 (163)	11.6 (99)	99
Ethyl methanesulfonate (no metabolic activation)	1	29.1 (99)	827 2813)	50 (170)	40
	4	35 (7)	3300 (660)	75 (15)	2.3
Ethyl methanesulfonate (metabolic activation)	1	34.4 (89)	1121 (2904)	161 (417)	30
	4	0 (0)	6300 (126)	3000 (60)	0.23
Trichloroethylene 1.11 mM	1	12.6 (104)	21.6 (178)	9.3 (77)	96
	4	7.8 (48)	15.9 (98)	7.5 (46)	72
Trichloroethylene 5.56 mM	1	12.1 (98)	13.1 (106)	9.4 (76)	94
	4	13.3 (74)	16.8 (94)	11.1 (62)	65
Trichloroethylene 111.1 mM	1	2552 (277)	3553 (323)	5313 (483)	< 1
	4	13700 (137)	22000 (220)	19800 (9198)	< 0.1
Trichloroethylene 222.2 mM	1	>23200 (232)	>29000 (290)	>25000 (250)	< 0.1
	4		0	>4100 (41)	< 0.1

*Numbers in parentheses refer to the number of revertant colonies counted per three plates.

(ineffective) and 111.1 mM (effective) were not tested. Furthermore, the composition of the trichloroethylene sample used in this study was not determined, and the possible contribution of contaminants to the observed mutagenic response cannot be ascertained nor ruled out (personal communication with R.C. Von Borstel, University of Alberta). Based on these results, one can state that the technical grade of trichloroethylene employed in these studies may have caused gene mutations after metabolic activation, but the fact that mutagenic events were observed only at concentrations resulting in high toxicity (< 99%) does not allow a conclusive evaluation to be made.

Margard (1978) compared the mutagenic properties of technical and purified grades of trichloroethylene in the Salmonella typhimurium/mammalian microsome assay using tester strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 in the presence and absence of a metabolic activation system (S-9 mix) from adult male Sprague-Dawley rats induced with Aroclor-1254. The trichloroethylene samples were added directly to cell suspensions prior to plating. Cells were incubated in plates containing histidine-deficient media for 48 hours at 37°C and scored for the number of colonies. The technical grade was contaminated with 0.08% (w/w) epichlorohydrin, 0.02% N-methylpyrrole, 0.13% N-propyl alcohol, 0.05% nitromethane, 0.10% 1,1,1-trichloroethane, and 0.45% butylene oxide. The purified grade contained no detectable epoxides nor other stabilizing ingredients (written communication with L. Schlossberg, Detrex Chemical Industries, Inc.). A concentration-related increase in the number of revertants was observed, as described in Table 11, in strain TA 1535 both with and without metabolic activation on exposure to technical grade trichloroethylene. This sample was not mutagenic in strains TA 98, TA 100, TA 1537, and TA 1538. The purified grade of trichloroethylene was not mutagenic for all strains. Trichloroethylene levels $\geq$ 0.1 ml/plate were toxic as indicated by reduced cell

TABLE 11. MUTAGENICITY OF TECHNICAL GRADE TRICHLOROETHYLENE
(adapted from Margard 1978)

		Activation			Nonactivation		
		TOXICITY	MUTAGENICITY		TOXICITY	MUTAGENICITY	
Tester Strain	Conc. ml/plate	Plate Count	Rel. Via.*	No. Rev.†	Plate Count	Rel. Via.*	No. Rev.†
TA 1535	1.0						
	0.5						
	0.1	27	0.14	58	22	0.14	58
	0.05	142	0.72	40	122	0.78	42
	0.01	185	0.94	17	149	0.95	21
	0	196	--	24	157	--	23
TA 100	1.0						
	0.5						
	0.1	53	0.19	184	66	0.28	173
	0.05	207	0.73	263	167	0.71	181
	0.01	255	0.90	123	232	1.0	136
	0	283	--	167	234	--	160
MUTAGENICITY OF PURIFIED GRADE TRICHLOROETHYLENE							
TA 1535	0.1						
	0.5						
	0.1	97	0.39	17	89	0.51	15
	0.05	172	0.70	19	121	0.69	20
	0.01	22	0.90	21	156	0.89	20
	0	246	--	22	175	--	19
TA 100	1.0						
	0.5						
	0.1	175	0.55	70	166	0.54	114
	0.05	175	0.55	142	162	0.53	158
	0.01	225	0.71	157	354	0.82	145
	0	317	--	154	305	--	158

*Relative viability
†Number of revertants

viability (49-86% killing). It has been reported that precautions were taken to inhibit evaporation of the test samples during treatment of the cells (Schlossberg, personal communication), but it was not specified in writing what those precautions were. Based on the observed toxicity, it is apparent that the cells were exposed to the test material. From the data presented, it is not clear how many plates were employed for each dose nor is it clear whether or not replicate testing was performed.

Somatic cell mutations in the stamen hairs of _Tradescantia_ clone 4430, an interspecific hybrid (_T. subacaulis_ x _T. hirsutiflora_), were utilized by Schairer et al. 1978 to assess the mutagenic potential of trichloroethylene. Although a positive result was observed, the purity and source of the sample tested were not provided and therefore it is not possible to ascribe with certainty the observed effects to the genotoxic action of trichloroethylene. A group of unrooted fresh cuttings containing young inflorescences possessing flower buds in a range of developmental stages was exposed to 0.5 ppm of trichloroethylene (and perhaps greater exposure levels too although these weren't specified) for 6 hours. Following treatment, the cuttings were grown in aerated Hoagland's nutrient solution and analyzed each day as they bloomed for 3 weeks after treatment. One hundred forty-eight mutations were observed out of 44,000 stamen hairs scored. The mutation frequency (± S.E./100 cells), after the negative control values (not reported) were substracted, was reported to be 0.112 ± 0.036 ($P < 0.01$). Based on the data given, however, this value can't be verified. Although it can't be determined whether or not concurrent positive controlled experiments were conducted, the mutation frequency (± S.E./100 cells) for 6-hour exposures to 5 ppm EMS and 75 ppm vinyl chloride were 1.012 ± 0.133 and 0.112 ± 0.046, respectively. It is concluded that the technical grade sample of trichloroethylene assayed in this study was mutagenic in _Tradescantia_.

Results of the above studies conducted in bacteria, yeast, and Tradescantia lead to the conclusion that the contamination of trichloroethylene with reactive epoxides must be considered when testing for mutagenicity and when evaluating the results from such tests. Although positive results were often obtained, one can't ascribe the mutagenic activity to trichloroethylene exclusively.

TESTING OF PURIFIED MATERIAL

Test results have been reported to show that trichloroethylene, free of detectable epoxides, is mutagenic. Greim et al. (1975) exposed Escherichia coli strain K12 to an analytical grade of trichloroethylene at a level of 3.3 mM (433.62 mg) during 2 hours of incubation at 37°C in the presence of metabolic activation with liver microsomes from phenobarbital-induced male mice (i.e., liver microsomes were used, not an S-9 microsome fractional). A chemical analysis of the test sample was not done by the investigators (written communication with D. Henschler, University of Wurzburg); however, the approximate composition of the trichloroethylene product would have been as follows: trichloroethylene, $> 99.9\%$; either ≤ 100 ppm diisopropylamine or ≤ 120 ppm of a combination of diisopropylamine, N-methylpyrrole, and 4-tert-butylphenyl as stabilizers; < 500 ppm of other chlorinated hydrocarbons combined (chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1,2-dichloroethylene, and tetrachloroethylene); no epoxides present at a detection level of 1 ppm (written communication with Dr. Fries and Dr. Koppe, E. Merck Company, the producer of the sample used in this study). Several loci in E. coli K12 were used to test for mutagenicity: back mutation at three loci, gal^+, arg^+, and nad^+, and the forward mutation MTR system which yields resistance to 5-methyl-DL-tryptophan. Survival of bacteria on complete medium at the trichloroethylene concentration used (3.3 mM) was 76 ± 4%. A weak mutagenic effect of trichloroethylene, expressed as percent of spontaneous mutation rate, was observed as follows: gal$^+$, 123 ± 23; arg$^+$, 232 ± 36; MTR, 114 ± 18; nad$^+$, 100. The reporting of the conduct of testing and observed results was inadequate. For instance, information concerning spontaneous mutation frequencies, number of replications, revertant count data, etc. for each test were not reported. Thus, the significance of the reported results can't adequately be assessed.

In tests conducted employing Salmonella tester strain TA 100 both with and without an exogenous S-9 metabolic activation, Bartsch et al. (1979) assessed the mutagenic potential of over 20 chemical substances including a trichloroethylene sample obtained from Merck, Darmstadt, Federal Republic of Germany. The sample was reported to be 99.5% pure and contained no detectable amounts of epichlorohydrin or 1,2-epoxybutane at levels $\geq$ 1 ppm. Experiments were conducted using a gaseous exposure assay in which petri dishes containing a lawn of TA 100 with or without S-9 mix were placed, uncovered, in 10-liter desiccators for various lengths of time at 37°C in the dark. Upon completion of the exposure period, the compound was removed, the plates covered, inverted, and placed in an incubator for 48 hours at 37°C and scored. Trichloroethylene was not found to be mutagenic under the conditions specified above (see below).

Concentration in Air (% v/v)	Time(h)	Revertants/plate +S9	Revertants/plate -S9
0		75	70
8	16	85	72
20	16	55 (toxic)	50 (toxic)

When the test conditions were modified such that plates containing a liver S-9 fraction from phenobarbital-treated mice, an NADPH-generating system, TA 100, and 0.1 mM EDTA were preincubated at 37°C for 4 hours and then exposed to 5%

trichloroethylene vapor in air for 2 hours, a nearly twofold increase over the spontaneous mutation frequency was observed (see blow).

Concentration in Air (% v/v)	Time(h)	Revertants/plate +S9	-S9
0		75	70
5*	2	100	80
5*	2†	135	70

*0.1 mM EDTA added to soft agar.
†Preinculated with S9, TA 100, and 0.1 mM EDTA for 4 hours.

Although the results of only one test were presented, it was stated that consistent twofold increases over the spontaneous mutation frequency were obtained. These results are consistent with those of other tests of purified samples of trichloroethylene conducted in sealed chambers to ensure exposure of the indicator organisms and suggest that trichloroethylene is a mutagen which requires metabolic activation, but the data do not allow a conclusive judgment to be made. In addition to testing trichloroethylene using TA 100, the ability of trichloroethylene to alkylate 4-(4')nitrobiphenyl (NBP) was assessed by adding concentrations ranging from 2.8 umol to 11 mmol trichloroethylene to 280 umol NBP and incubating in 3 ml of acetone under oxygen at 37°C. After 30 minutes, absorption spectra between λ = 450-600 nm were recorded. No NBP adducts with absorbancies between these wavelengths were obtained for trichloroethylene. However, the authors speculate that "a low rate of conversion of trichloroethylene into mutagens or a rapid inactivation of microsomal enzymes through irreversible binding of its metabolites (Uehleke and Poplawski-Tabarelli 1977) may explain its weak 'mutagenic' effect in S. typhimurium, which was observed only under special assay conditions. It may also explain our inability to trap the oxirane intermediate with NBP."

Baden et al. (1979) evaluated the mutagenic properties of trichloroethylene in Salmonella typhimurium strains TA 100 and TA 1535 both with and without S-9 metabolic activation from PCB-induced male Sprague-Dawley rats. The trichloroethylene sample was > 99.5% pure, contained no detectable epoxides, and was stabilized with 0.1% (w/w) thymol (personal communication with M. Kelley, V. A. Hospital, Palo Alto, CA). Cultures were exposed to trichloroethylene vapor concentrations ranging from 0.1 to 10.0% (± 10% tolerance) in a desiccator for 8 hours at 37°C with further incubation without treatment for 40 hours before counting of colonies. The experiment was repeated, and each test concentration was evaluated with triplicate assays. Trichloroethylene vapor produced a statistically significant ($P < 0.01$) increase in the number of revertant colonies to approximately 30% above background (149 ± 12, n=15) at concentrations of 1% (196 ± 13, n=15) and 3% (194 ± 12, n=15) in the TA 100 strain with metabolic activation. In another experiment, liquid trichloroethylene was directly added to bacterial cell cultures for incubation at 37°C for 2 hours, and concentrations in the gas phase above the incubator, which were the same as those in the desiccator experiment, were monitored to show variation of ≤ 10%. These cultures were plated and incubated for 2 days at 37°C for counting of colonies. Trichloroethylene was not found to be mutagenic in this experiment, but trichloroethylene concentrations were apparently not monitored after the initial 2-hour incubation period to ensure exposure throughout the entire 2-day incubation period. Toxicity data were not reported and can't be used to determine whether or not the target cells were actually exposed to a sufficiently high concentration of the test material, but the weakly positive response obtained in tests conducted in desiccators suggests that trichloroethylene was mutagenic under these conditions but the results are equivocal. It should be noted that there was no thymol control performed to rule out the possibility that it was causing the weak positive response.

Simmon et al. (1977) also reported that reagent grade trichloroethylene, with no detectable epoxides, was weakly mutagenic in _Salmonella typhimurium_ strain TA 100. The trichloroethylene sample was 99.85% pure, and contaminants included 0.09% chloroform, 0.02% tetrachloroethylene, 0.01% pentachlorobutadiene, and 0.03% hexachlorobutene; epoxides were not detected (personal communication with R. Spanggord, SRI International). Plates of _Salmonella typhimurium_ strain TA 100 cells with and without metabolic activation were exposed to trichloroethylene vapor evaporated from a known amount of liquid in a desiccator for 7 hours at 37°C. The cells were subsequently incubated for 40 more hours without treatment at 37°C before counting. Trichloroethylene was detected as mutagenic only with metabolic activation. The number of revertants per plate was increased above the spontaneous control value of 140 by trichloroethylene treatment as follows: 180, 230, 240, and 200 revertant counts at 500, 1000, 1500, and 2000 ppm trichloroethylene vapor, respectively, in the presence of liver microsomes from B6C3F1 male mice and 160, 210, 200, and 170 at 500, 1000, 1500, and 2000 ppm trichloroethylene vapor, respectively, in the presence of a metabolic activation system derived from PCB-induced male Sprague-Dawley rats. The revertant counts were extrapolated from Figure 22 of the paper. The results of these assays were reproduced several times (personal communication with V. Simmon, Genex Corp), but no estimation of the variance of the results (stated to be low) was presented and no data are presented to allow its calculation. Although less than a twofold response was obtained, the dose-related increase observed with metabolic activation suggests that trichloroethylene is metabolized to a mutagenic substance(s) under these conditions of test.

An increase in point mutations and gene conversion by a certified ACS reagent grade of trichloroethylene in _Saccharomyces cerevisiae_ strains D4 and D7 was observed by Bronzetti et al. (1978) in cell suspension and in a host-mediated assay. The authors did not report a detailed chemical analysis of the sample; however, a description of the trichloroethylene product obtained by personal communication with Fisher Scientific Company, the producer of the test sample used in this study, indicates a purity of > 99.5% and stabilization with either 25 ppm diisopropylamine or 20 ppm triethylamine. Mitotic gene conversion at the ade and trp loci can be detected with the D4 strain, and the D7 strain can detect mitotic gene conversion at the trp locus, point (reverse) mutation at the ilv locus, and mitotic recombination at the ade locus. In the cell suspension experiment, strain D7 cells were incubated in the presence of trichloroethylene with or without a metabolic activation system derived from livers of uninduced male CD-1 mice for 4 hours at 37°C before plating on selective media for counting of ilv revertants and ade and trp convertants and on complete medium for survivor counts. The results are presented in Table 12.

Yeast cell culture was instilled into the retro-orbital sinus of male adult CD-1 mice for the host-mediated assay (Bronzetti et al. 1978). Two experiments were included in this assay: an acute experiment in which a single gavage dose of 400 mg trichloroethylene/kg was given immediately after introduction of strain D4 or D7 cells and a subacute experiment in which pretreatment gavage dosages of 150 mg trichloroethylene/kg were made 5 days per week for 22 treatments followed by a gavage dose of 400 mg trichloroethylene/kg immediately after administration of D4 strain cells on the day of the assay. Mice were sacrificed 4 hours following introduction of the yeast cells, and liver, lungs, and kidneys were removed for homogenization and subsequent plating as described for the suspension assay. Assays in this study were done in triplicate or quadruplicate, and the data are presented in Table 12 as mean ± S.E.

The results of Bronzetti et al. (1978) were as follows. In the absence of

TABLE 12. MUTAGENICITY OF REAGENT GRADE TRICHLOROETHYLENE IN YEAST
(adapted from Bronzetti et al. 1978)

Suspension Tests Strain D7

	Locus		
Concentration (mm)	ilv 1^+ revertants/10^6	trp$^+$ convertants/10^6	% Survival
Without Metabolic Activation			
0	0.75 ± 0.12	17.7 ± 2.99	100
10	0.85 ± 0.15	12.3 ± 0.67	87.5 ± 1.0
40	0.87 ± 0.06	13.2 ± 1.7	64.1 ± 2.1
With Metabolic Activation			
0	0.85 ± 0.06	16.3 ± 0.42	100
10	1.16 ± 0.16	17.3 ± 1.9	81.6 ± 2.9
20	1.77 ± 0.12	22.3 ± 1.1	67.1 ± 2.8
30	2.24 ± 0.17	33.8 ± 5.7	55.5 ± 1.7
40	3.23 ± 0.32	41 ± 2.6	50.3 ± 1.4

(continued on the following page)

TABLE 12. (continued)

Host-Mediated Assay

Strain	Organ	Treatment	No. of Mice	trp+ convertants/10^6	Ade convertants/10^6	ilv 1^+ convertants/10^6
Acute Exposure (400 mg/kg) Mean ± SD						
D4	Liver	Control	3	13.6 ± 6.0	17.3 ± 2.4	
		TCE	4	36.0 ± 7.4	31.9 ± 12.3	
	Lungs	Control	3	13.6 ± 2.8	22.2 ± 5.7	
		TCE	3	22.6 ± 3.3	27.8 ± 5.3	
	Kidneys	Control	3	102.6 ± 30.1	53.9 ± 55.4	
		TCE	3	253.6 ± 29.2	105.6 ± 55.4	
D7	Liver	Control	3	28.2 ± 4.6		0.8 ± 0.4
		TCE	4	106.9 ± 36.3		5.6 ± 5.2
	Lungs	Control	2	44.4 ± 0.3		0.8 ± 0.02
		TCE	3	36.1 ± 8.8		4.0 ± 1.9
	Kidneys	Control	2	60.8 ± 17.2		2.1 ± 0.2
		TCE	4	259.0 ± 101.7		7.9 ± 3.7
Chronic Exposure (3700 mg/kg total dose)						
D4	Liver	Control	4	13.7 ± 5.0	13.0 ± 2.9	
		TCE	4	47.5 ± 5.9	62.3 ± 11.0	
	Lungs	Control	4	13.0 ± 0.3	12.0 ± 3.0	
		TCE	4	20.1 ± 6.5	23.3 ± 6.5	
	Kidneys	Control	3	18.6 ± 5.0	12.6 ± 4.7	
		TCE	4	187.9 ± 58.3	37.7 ± 44.2	

metabolic activation, 10 mM and 40 mM (1,314 mg and 5,256 mg) concentrations of trichloroethylene effected a dose-related reduction in cell viability in the cell suspension system but did not induce a mutagenic response. Survival was reduced to approximately 87% with the 10 mM dose and 65% with the 40 mM dose compared to 100% for the controls. Trichloroethylene did produce concentration-dependent increases in point mutation and gene conversion frequencies in the cell suspension experiment with metabolic activation at doses of 10, 20, 30, and 40 mM with metabolic activation. No positive controls were reported so one cannot determine from the data presented whether or not the test system was working efficiently, but the positive response obtained with activation suggests that it was.

In the host-mediated assays, increased gene conversion frequencies in strain D4 yeast cells at both the ade and trp loci were observed after treatment of the mice with trichloroethylene. The largest increases were found in liver and kidney, and gene conversion frequencies were higher with subacute dosing compared to acute dosing.

Like Bronzetti et al. (1978), Callen et al. 1980 also employed a trichloroethylene sample obtained from the Fisher Scientific Company. They used Saccharomyces cerevisiae strains D4 and D7 to assess trichloroethylene's genetic activity and strain D5 intact yeast cells or microsomal suspensions to indirectly study its metabolism. Gene conversion at the trp 5 locus, mitotic recombination at the ade 2 locus and gene conversion of the ilv 1 locus of strain D7 were assessed by exposures of the cells to 0, 15, and 22 mM solutions of trichloroethylene in suspension at 37°C for 1 hour followed by plating on appropriate media, allowing for expression of genetic activity and, finally, scoring. Treatment of strain D4 under similar conditions reportedly resulted in only marginal increases in gene conversion but the data were not presented for analysis. The results of the testing of strain D7 presented in Table 13, demonstrate significant increases in gene conversion and mitotic recombination and a slight increase in reverse mutations at the ilv 1 locus over the spontaneous control values at 15 mM trichloroethylene and a severely toxic effect at 22 mM. Thus, it is apparent that trichloroethylene entered the cells and exerted a genotoxic effect.

To address the issue of whether or not trichloroethylene is metabolized by yeast, test material was added to whole cell suspensions and the spectrum from 410 to 550 nm was recorded (Callen et al. 1978). Trichloroethylene gave a peak at 450 and 505 nm. The peak at 450 nm appeared to reach a maximum after roughly five minutes and after this period an additional peak at 423 nm was observed. This spectrum is similar to that observed in mammalian microsomes. These results indicate that trichloroethylene does enter the yeast cells and is metabolized by a yeast cytochrome P-450-dependent monooxygenase system.

Tests to determine the mutagenicity of trichloroethylene have not been limited to prokaryotes and microbial eukaryotes. Abrahamson and Valencia (1980) fed 1000 ppm trichloroethylene in 0.2% sucrose for 72 hours to male Canton-S (wild type) Drosophila melanogaster, and subsequently mated 102 of these flies with female FM6 (a marked and balanced X-chromosome) flies to screen descendents for mutagenicity using the sex-linked recessive lethal test. Ninety-six of the treated males were fertile. The trichloroethylene feeding solution was provided in a glass shell vial containing filter material saturated with feeding solution and the feeding solution was renewed twice daily. However, because of the volatility and slight solubility of trichloroethylene in aqueous media, the actual dose given to the flies might have been much less than expected. Treated and untreated control males were mated individually with three females each and then transferred without anesthetization to fresh females at 2 to 3 day

TABLE 13. MUTAGENICITY OF TRICHLOROETHYLENE IN SACCHAROMYCES CEREVISIAE D7 (adapted from Callen et al. 1980)

	Survival		trp 5		ade 2				ilv 1	
Concentration (mM)	Total Colonies	%	Total No. Survivors Convertants	Convertants 10^6 Survivors	Total Twin Spots	Mitotic Recombination/ 10^6 Survivors	Total Genetic Alterations	Frequency 10^6 Survivors	Total Revertants	Revertants 10^6 Survivors
0	1201	100	171	14	1	250	13	3300	43	3.6
15	802	67	610	76	16	3600	53	12000	59	7.4
22	3	0.3								

intervals until a total of four broods of progeny were produced. The fertile females were left in the vials for 1 week. In a simultaneous experiment, the mutagenicity of trichloroethylene was tested by injecting 0.3 ul trichloroethylene into the abdomen of the male flies before mating. A technical grade trichloroethylene sample from Allied Chemical Co. was used; however, the purity and composition of the sample was not ascertained (personal communication with L. Valcovic, Food and Drug Administration, which sponsored this study, and Allied Chemical Co.).

Trichloroethylene was not demonstrated to be mutagenic in the above test. The frequency of lethals (number of lethals/number of chromosomes tested) was 0.21% (19/9248) in the feeding experiment, 0.17% (4/2344) in the injection experiment, 0.31% (31/10009) in concurrent controls, and 0.24% (230/94491) in historical controls used in the testing laboratory for 2 years prior to completion of this study. The negative result obtained in this test indicates that either the test substance did not reach the target site (DNA in cells of germinal tissue) or that trichloroethylene is not mutagenic. There is a possibility that much of the trichloroethylene may have boiled off before being eaten by the flies. This possibility and the fact that the compound or an active metabolite would have to reach germinal tissue to exert a mutagenic effect may explain the negative results obtained.

The mutagenicity test of particular importance to the assessment of the carcinogenicity of trichloroethylene is the positive mouse spot test reported by Fahrig (1977). Virgin females of the inbred C57BL/6J Han strain (a/a, wild type) were mated with males of the Han-rotation bred T-stock (a/a=non-agouti, b/b=brown, c^{ch} p/c^{ch} p=chinchilla and pink-eyed dilution; d se/d se=dilute and short ear; s/s=piebald spotting) to yield embryos of the genotype a/a, b/+, c^{ch}p/+ +, d se/+ +, s/+ (black coat, dark eyes). Each male was mated with two females. On day 11 of gestation, a single intraperitoneal injection of trichloroethylene (99.5% pure) was made into 50 and 26 females at doses of 140 and 350 mg/kg, respectively. Litters were born on day 21 of gestation, and pups were examined for color spots, the mutagenic end-point, twice weekly between 2 and 5 weeks of age.

The number of offspring (number of litters) surviving for examination was 144 (23) in the untreated control group, 145 (38) in the low dose group, and 51 (13) in the high dose group. Coat color (brown or gray) spots were found in no concurrent control pups, 2/794 historical control pups (0.25%), two low-dose pups (1.3%), and two high-dose pups (3.92%). Known mutagens including ethyl methanesulfonate were positive in this study (26% at 0.9 mM EMS). Results of this study indicate that trichloroethylene or an active metabolite is able to produce mutations in somatic tissue of an intact mammal. If, as is generally believed, somatic cell mutagenesis is an initiating event which can lead to cancer, the results of this test provide supporting evidence for the carcinogenicity of trichloroethylene. Impurities detected in this test sample were 60 to 150 ppm ethanolamine, 0.0003% chloride (Cl_2), and 0.0001% free chloride (Cl^-) (personal communication with R. Fahrig, University of Tubingen, West Germany).

Slacik-Erben et al. (1980) assessed the ability of 0, 50, 202, and 450 ppm (0, 273, 1104, and 2459 mg/m^3) of trichloroethylene in air to cause dominant lethal mutations in NMRI-Han/BGA mice. The sample of trichloroethylene contained 99.5% 1,1,2-trichloroethylene, 100 mg/l triethanolamine, 5 ppm 1,2,-dichloroethylene, 9 ppm cis-1,2-dichloroethylene, 80 ppm 1,1,1-trichloroethylene, 120 ppm carbon tetrachloride, 10 ppm 1,1,2-trichloroethane, and 815 ppm tetrachloroethylene. Fifty males per dose were exposed for 24 hour, in a 1000 l volume glass chamber equipped with a ventilator. The temperature was kept between 22-24°C and the humidity between 70-80%. For rapid equilibration, the calculated volume of liquid trichloroethylene was placed in the chamber and the mixtures of trichloroethylene in air were streamed through the chamber at a rate of 200 (50 and 202 ppm) and 300 (450 ppm) 1/h. After the 24-hour exposure period, the male mice were mated with one untreated NMRI-Han female for four days. At this time, the mated females were replaced by fresh virgin females who were kept for four days and then replaced, etc. until each male had been mated singly to 12 females for four days each (up to 48 days after treatment). By this mating regimen all stages of spermatogenesis were sampled from mature sperm to gonial cells. Each pregnant female was sacrificed by cervical dislocation, dissected, and examined for dead and living implants. The results of the testing are presented in Table 14. Under conditions of this test, trichloroethylene was not detected as mutagenic, but there are several important factors to consider with respect to this finding. The males exhibited no visible effects during the exposure, and doses up to 450 ppm did not alter the fertilization rate. Thus, the doses selected for test may not have approached the maximum tolerated dose. Even if the maximum tolerated dose was approached, the dominant lethal test is recognized not to be a sensitive test for detecting mutagens (Russell and Matter 1980) due to the high spontaneous level of dominant lethal events. The spot test, on the other hand, has been reported to be a more sensitive test than either the dominant lethal assay or the host-mediated assay employing yeast as the indicator organism (Fahrig 1978). Although trichloroethylene did not induce a significant increase in dominant lethal mutations in the testing by Slacik-Erben et al. 1980, these results are not considered to diminish the positive findings of mouse spot test.

TABLE 14. MUTAGENICITY OF TRICHLOROETHYLENE IN THE DOMINANT LETHAL ASSAY (adapted from Slacik-Erben et al. 1980)

Dose (ppm)	Female Mated	Female % w/Implants	Total Implants	Total Dead Implants	Total Live Implants	Live Implants Female	% Dominant Lethal Factors*
200 1/h							
0	498	89.6	5578	443	5135	11.5	
50	482	90.1	5360	479	4881	11.2	2.6
202	493	91	5703	531	5172	11.5	0
300 1/h							
0	503	93.4	6142	502	5640	12.0	
450	488	92.4	5927	533	5394	11.97	0.25

*Dominant lethal factors (F_L)

$$F_L\% = 1 - \frac{\text{(live implants per female of test group)}}{\text{(live implants per female of the control group)}} \times 100$$

Metabolites

Waskell (1978) investigated the mutagenic properties of commonly used volatile anesthetics and several of their known metabolites in Salmonella typhimurium strains TA 100, TA 98, TA 1535, and his G. Several of these compounds were also tested in Salmonella typhimurium strains TS 24 rec A, TA 2322 pol A, and TA 1950 uvrB, strains deficient in their capacity to repair DNA. Volatile compounds were tested in closed containers to which an amount of liquid was added which would volatilize to yield the desired vapor concentration. The assays were done according to standard procedures described by Ames et al. (1973, 1975) in the presence or absence of a metabolic activation system containing hepatic microsomes from male Sprague-Dawley rats. In the repair test, the test chemical was placed on a sterile filter paper disc which was placed on the bacterial lawn; the plates were incubated at 37°C for 24 hours and the zone of killing measured.

Anesthetic-grade trichloroethylene from Ayerst was used, and this product would have been of an approximate minimum purity of 99.5% with stabilization by 0.1% thymol (personal communication with Ayerst). The 1,1,1-trichloroacetic acid sample obtained from Matheson, Coleman, and Bell was $\geq$ 99.0% pure (personal communication with Matheson, Coleman, and Bell). Chloral hydrate was recrystallized from chloroform six times to achieve a purity of $\geq$ 99.0%, and the purity of trichloroethanol was not determined (personal communication with L. Waskell, V. A. Hospital, San Francisco, CA).

Nonvolatile compounds were tested at maximum nontoxic quantities per plate. With respect to trichloroethylene metabolites, 7.5 mg/plate trichloroethanol and 0.45 mg/plate trichloroacetic acid were not mutagenic in strains TA 98 and TA 100; however, 10 mg/plate chloral hydrate, though inactive in strain TA 98, increased the number of revertants above control (200) by 121 with metabolic activation and 89 without metabolic activation in strain TA 100. These strains were responsive to the positive control chemicals diethyl sulfate and 2-aminofluorene in this test.

Further testing was done to evaluate the dose-response of chloral hydrate-induced mutagenesis in strain TA 100. The number of revertants per plate in the control group were 190 and 210 with and without metabolic activation, respectively. Although they did not result in a mutation frequency greater than twofold above the spontaneous level, doses of 0.5 mg/plate to 10.0 mg/plate chloral hydrate resulted in statistically significant ($P < 0.01$) increases in the number of revertants per plate above control values: 255 to 260 at 0.5 mg; 285 to 290 at 1.0 mg; 300 to 320 at 5.0 mg; and 290 to 310 at 10.0 mg. The dose-responses obtained with and without metabolic activation were equivalent.

Unlike the report by Simmon et al. (1977), trichloroethylene exposure concentrations up to a maximum of 10% for 48 hours was reported by Waskell (1978) not to produce a mutagenic effect in strains TA 100 and TA 98 with and without metabolic activation. The assays with trichloroethylene were done in duplicate but a different testing protocol was employed compared to the one employed by Simmon. Trichloroethanol (31 mg) was evaluated in the repair test and found to be negative. The weak positive response with chloral hydrate, a metabolite which is found in rats and man, suggest that exposure of mammals to trichloroethylene may result in the induction of mutation via metabolism of the compound to chloral hydrate.

Chromosomal Aberrations

White et al. (1979) evaluated the ability of anesthetic grade trichloroethylene (source and purity not reported) to cause sister-chromatid exchanges (SCE) in Chinese hamster ovary cells. Trichloroethylene was administered as a gas to exponentially growing cells at 0.17% (MAC value) plus 19% oxygen, 5% carbon dioxide and 75.83% nitrogen for 1 hour in the presence of S-9 mix prepared from Aroclor 1245-treated male rats. After 1 hour of exposure, the concentration of trichloroethylene gas in the flask was measured by gas chromotography and found to be 59% of that delivered. After treatment, the cells were allowed to go two rounds of cell division in the presence of bromodeoxyuridine (BrdU). Subsequently, chromosome preparations were made and stained with Giemsa. One-hundred control and 100 treated metaphases were scored for SCEs by a single observer and the observed number of SCEs/chromosome were 0.536 ± 0.018 and 0.514 ± 0.018, respectively. Under the conditions of this test, anesthetic grade trichloroethylene did not yield an increased incidence of SCEs. However, it should be noted that the conditions of the test may not have provided an adequate assessment of trichloroethylene's ability to cause sister chromatid exchanges. In the first place, the dose administered may have been too low. Only 59% of the delivered dose remained 1 hour after exposure so the cells were only exposed to 0.1003% trichloroethylene rather than 0.17%. There was no indication of toxicity in the treated cells further suggesting that they did not receive an adequate dose. In the second place, no concurrent positive controls were employed to ensure that the test system was working properly. These deficiencies reduce the weight of the negative response obtained by White et al. (1979).

Konietzko et al. (1978) studied the incidence of chromosome aberrations (structural and numerical) in 28 chemical workers engaged in the manufacture of trichloroethylene. The workers ranged in age between 23 and 67 years with an average age of 42.5. Their exposures ranged in duration from 1 to 21 years; the average length of exposure was approximately 6 years. Since many of the workers had been exposed over a period of many years, it was not known what contaminants and stabilizers they may have been exposed to. Thus, the study could only permit conclusions to be drawn concerning the long-term effects of exposure to technical grade trichloroethylene, not to the purified material. A control group of 10 healthy young men working in the Institute of Human Genetics where the study was conducted and ranging in age from 15 to 45 years (average age 28.3 years) was selected for comparison. However, this group was not a matched control group. The exposed group had a frequency of 10.96 ± 4.4% (X ± SD) hypodiploid cells compared to the control group which had a frequency of hypodiploid cells of 6.5 ± 3.2%. Concerning chromosome breaks/100 cells the frequencies were 3.1 ± 3.6 and 0.6 ± 0.69, respectively. Since the control group was not a matched control group and since the mean frequency of chromosome breaks observed in the exposed workers was within the normal range, the increased incidence of chromosome breaks was not considered significant. It was thought appropriate to further investigate factors which may have contributed to the increased incidence of hypodiploid cells. Based on the frequency of hypodiploid cells from the controls, it was calculated that individuals possessing > 13% hypodiploid cells were abnormal. Workers were divided according to whether or not they possessed > 13% hypodiploid cells and and these groups of workers were subsquently further characterized (Table 15). Nine individuals had greater than 13% hypodiploid cells. As noted in the table, these individuals worked under significantly higher daily peak concentrations of trichloroethylene (as measured on three occasions over a 2-week period for 8 hours each time) than did their fellow workers. These measured exposure levels were extrapolated to estimate previous years of exposure because no technical changes had been made in the plants. This report provides suggestive evidence that technical grade trichloroethylene causes chromosomal loss or nondisjunction in humans.

Several other reports bear on the potential of trichloroethylene to cause

TABLE 15. NUMERICAL CHROMOSOME ABERRATIONS IN TRICHLOROETHYLENE WORKERS
(adapted from Konietzko et al. 1978)

Parameter	> 13% Hypodiploid Cells (n* = 9) Mean Value (X)	> 13% Hypodiploid Cells (n* = 9) Standard Error (S_x)	< 13% Hypodipoloid Cells (n* = 18) Mean Value (X)	< 13% Hypodipoloid Cells (n* = 18) Standard Error (S_x)
Daily average concentration (ppm)	75.0†	17.4	61.4	23.1
Average maximum concentration (ppm)	205.8†	96.3	115.7	57.6
Exposure (years)	5.9	4.8	5.9	5.1
Total dose (ppm x day)	0.55×10^6	0.72×10^6	0.31×10^6	0.39×10^6
Age (years)	38	9.4	40.4	9.7
Alcohol (g/day)	18.9	29.3	33.6	37.5
Nicotine (cigarettes/day)	12.8	12.7	10.1	10.2

*n = Number of individuals studied.

†Statistically significant ($P < 0.05$).

nondisjunction. These reports describe the ability of anesthetic gases (trichloroethylene and structurally-related chemical substances) to alter cell growth, mitosis of polymerization of microtubules (Sturrock and Nunn 1975, Ogli 1977, and Okuda and Ogli 1976). Trichloroethylene was found to alter cell growth but was not used in the studies which were designed to detect altered mitosis or microtubule polymerization. It is, however, structurally related to compounds which do cause these effects. Thus, although the data reported don't allow one to conclude that trichloroethylene causes alterations on mitosis or microtubule polymerization, they suggest there is a potential problem which deserves further investigation. Given trichloroethylene's potential for altering microtubule polymerization and the lack of cytogenetic studies which have been performed to assess its chromosome damaging potential, it would be appropriate to test for its ability to cause chromosome damage (both structural and numerical) in experimental mammals (in vivo and in vitro). Special care must be exerted to utilize a highly purified sample of trichloroethylene and to take precaution to ensure exposure to target cells. It would also be appropriate to assess the ability of the trichloroethylene to alter microtubule polymerization.

Although not bearing on the ability of trichloroethylene to cause cancer via the induction of somatic cell mutations, the report by Land et al. (1979) that anesthetic grade trichloroethylene induces a statistically significant increase in sperm morphological abnormalities in mice may indicate that the compound or an active metabolite reaches the gonads and has the potential to cause germ cell mutations. Thirteen-week-old (C57BL/C3H)F_1 male mice were randomized and three exposure groups comprised of five individuals per exposure group were placed in cages. These groups were exposed to air, 0.02% and 0.2% trichloroethylene, respectively, 4 hours/day, for 5 consecutive days.

Twenty-eight days after the first day of exposure, the animals were sacrificed. Both cauda epididymides were removed, minced, strained, and stained in 1% Eosin Y. Slides were prepared, mounted, coded, and evaluated by scoring 1000 sperm/slide at 400 X magnification. Data were recorded as percent abnormal sperm. The means were calculated for each group and compared with controls using the sample t-test. As noted below, trichloroethylene resulted in statistically significant elevations in the percentage of morphologically abnormal sperm.

Agent	Concentration %	Number of Animals Studied	% Abnormal Sperm ± S.E.
Air		15	1.42 ± 0.08
Trichloroethylene	0.02	5	1.68 ± 0.17
	0.2	5	2.58 ± 0.29*

*Statistically significant difference compared to controls ($P < 0.001$).

One concludes from this study that after prolonged exposure to a high concentration of trichloroethylene, damage to the early spermatocyte during the period of meiotic cell division occurred to a small but significant percentage of cells.

In summary, trichloroethylene has been tested for its ability to cause gene mutations in bacteria, yeast, *Drosophila*, mice, and *Tradescentia*. It has been tested for its ability to cause primary DNA damage using the SCE technique and it has been studied for its ability to cause chromosome damage in humans. Additionally, tests have been performed addressing its effects on mitosis and its ability to reach the gonads of an intact mammal. Because trichloroethylene is highly volatile and not very soluble, it has been difficult to adequately test this material. Several investigators reporting negative results possibly failed to take these properties into account and, therefore, the concern is raised that the target cells were not adequately exposed. Another factor which makes it difficult to assess the significance of the reported results is that trichloroethylene is unstable and stabilizers must be added to it to prevent its decomposition. In the past, compounds known to be potent mutagens, such as epichlorohydrin, have been used as stabilizers. Therefore, positive results reported in papers not specifying the purity of the tested material and its contaminants must be viewed with caution. These factors have rendered many of the reports concerning an assessment of the genotoxicity of trichloroethylene inappropriate. Several reports have indicated weak but positive responses in tests conducted with highly purified samples of trichloroethylene using bacteria, yeast, and more importantly intact mice. Thus, based on the weight of evidence available at this time, trichloroethylene is evaluated as being a weak mutagen. Since the metabolism and pharmacokinetics of this compound in humans results in metabolic products which can interact with DNA (e.g., chloral hydrate) as is the case for bacteria, yeast, and mice, it is conducted that it will likely cause mutagenic effects in humans as well, if it reaches DNA within exposed cells. Furthermore, if the compound or an active metabolite reaches the gonads, as is the case for mice, it may result in the production of heritable mutations.

CELL TRANSFORMATION

Price et al. Study

Price et al. (1978) exposed Fischer rat embryo cell cultures (F1706, subculture 108) to trichloroethylene liquid at concentrations of 1.1×10^3 and 1.1×10^4 uM (1 uM = 131.4 ug) for 48 hours. Trichloroethylene was diluted with growth medium to yield the appropriate doses. The trichloroethylene sample, obtained from Fisher Scientific Co., was $\geq$ 99.9% pure and would have been stabilized with 20 ppm triethylamine or 25 ppm diisopropylamine (personal communication with Fisher Scientific Co.). The cells were grown in Eagles minimum essential medium in Earle's salts supplemented with 10% fetal bovine serum, 2mM L-glutamine, 0.1 mM nonessential amino acids, 100 U penicillin, and 100 ug streptomycin per ml. Quadruplicate cultures were treated at 50% confluency with each dose. After treatment, cells were cultured in growth medium alone at 37°C. Transformation of cells treated with either dose level of trichloroethylene was observed by 44 days of incubation and was characterized by progressively growing foci composed of cells lacking contact inhibition and orientation. There was no transformation of cells grown in medium alone or in the presence of a 1:1000 acetone concentration by subculture 9 (81 days of incubation). Nine and eight microscopic foci per three dishes with the low and high trichloroethylene dose, respectively, were found in dishes inoculated with 50,000 cells from cultures treated four subcultures earlier and held for 4 weeks at 37°C in a humidified CO_2 incubator prior to staining. According to the authors, this cell line, at the subculture used, did not require addition of exogenous murine type "C" RNA virus and was free of any known infectious virus; however, these cells did contain genetic information of the Rauscher leukemia virus. Nonetheless, while it is understood that the genome for the Rauscher leukemia virus exists in the Fischer F1706 rat embryo cell line, the positive result with trichloroethylene is not considered minimized since the mode of action for its cell transformation effect is not known.

Subcutaneous injection of cells treated with trichloroethylene five subcultures earlier produced local fibrosarcomas in 13/13 and 12/12 newborn Fischer 344 rats within 48 to 55 days following treatment. The ability of cells grown in growth medium alone to induce local fibrosarcomas was not determined; however, this tumor type did not develop in rats given cells grown in the presence of a 1:1000 concentration of acetone. Exposure of cells to 3.7×10^{-1} uM 3-methylcholanthrene produced 124 microscopic foci per three dishes in the inoculation test described above by 37 days of incubation and local fibrosarcoma in 12/12 rats by 2 days following subcutaneous injection of cells. The exposure level of 3-methylcholanthrene was attained by an initial dilution in acetone to 1 mg/ml followed by further dilution in growth medium to 0.1 ug/ml. The authors did not attempt a repeat of this study (personal communication with P. L. Price, Center for Disease Control, Atlanta).

Although transformation of F1706 cells in the presence of trichloroethylene was observed by Price et al. (1978), the effect was small compared to that produced by the positive control agent 3-methylcholanthrene. The response to trichloroethylene may reflect an ability of this cell line to convert trichloroethylene to active metabolites, and further evaluation of the effect of trichloroethylene through active metabolites possibly could have been made by additional testing with an exogenous metabolic activation system. Additionally, cells were exposed to liquid trichloroethylene diluted in growth medium; therefore, since trichloroethylene is a volatile substance, exposure of cells to trichloroethylene as a vapor could have indicated how different exposure conditions possibly could have influenced its ability to transform F1706 cells.

Styles Study

Styles (1979) reported an investigation on trichloroethylene in a cell transformation system with BHK cells, using growth in semi-solid agar as an endpoint, as part of a larger study (Purchase et al. 1978) done to screen chemicals for carcinogenic potential. The BHK-agar transformation assay technique used has been described by Styles (1977) and Purchase et al. (1978). In this study, baby Syrian hamster kidney (BHK-211Cl 13) cells were exposed to five different doses of test substance in vitro in serum-free liquid tissue culture medium either in the presence or absence of rat liver microsomal fraction and cofactors (S-9 mix; Ames et al. 1975).

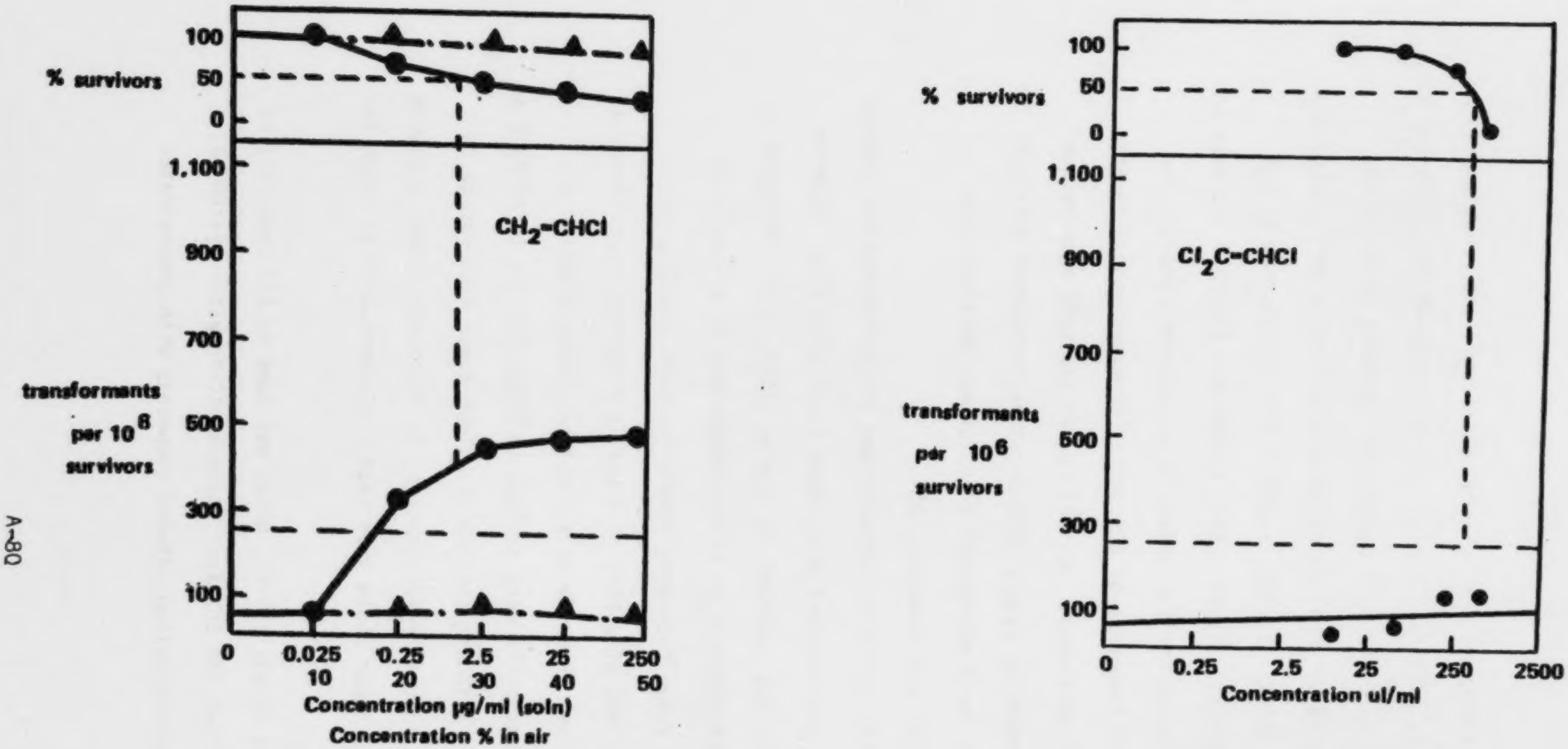
Cells were grown and maintained in Dulbecco's modification of Eagles medium in an atmosphere of 20% CO_2 in air. Cells were maintained at 37°C until confluent; subsequently, the cells were trypsinized and resuspended in fresh growth medium. Resuspended cells were grown until 90% confluent for transformation assays or 100% confluent for stock. Only cells with normal morphology were used for assays. To miminize spontaneous transformation frequency, cells were obtained at low passage, grown to 90% confluency, and frozen in liquid nitrogen. Cells were thawed at 37°C in growth medium for further use.

Test compounds were dissolved in DMSO or water as appropriate. Each dose was tested in replicate assays. Cells incubated until 90% confluency were trypsinized and resuspended in Medium 199 at a concentration of 10^6 cells/ml. Resuspended cells (10^6) were incubated with test chemical and with or without S-9 mix were incubated at 37°C for 4 hours. To evaluate compound vapors, cells (2.5×10^5) were plated and incubated until 90% confluent at which time growth medium was replaced with medium 199 and S-9 mix added as appropriate. Duplicate plates were exposed to test chemical vapor at

37°C for 3 hours. After treatment, cells were centrifuged and resuspended in growth medium containing 0.3% agar. Survival after treatment was estimated by incubating 1,000 cells at 37°C for 6 to 8 days for counting of colonies. Transformation was evaluated by counting colonies after cells were plated and incubated for 21 days at 37°C. The dose-response for transformation was compared to that for survival. Styles (1977) accepted a fivefold increase in transformation frequency above control values as a positive result. The spontaneous transformation frequency of BHK cells (72 experiments) in this study was 50 ± 16 per 10^6 survivors. Suitability of the soft agar medium for colony growth was checked by assays with polyoma-transformed BHK-21/Cl 13 cells or Hela cells. The liver microsomal fraction was obtained from Sprague-Dawley rats induced with Arochlor 1254.

Data reported by Styles (1979) on unstabilized trichloroethylene (source and purity not reported) are compared with those found with vinyl chloride (ICI Mond Division; purity not reported) by Styles (1979, 1977) in Figure 2. Results were negative with exposure to trichloroethylene as a liquid in culture medium in a dose range including levels in which toxicity was observed. Vinyl chloride was effective as a gas in producing transformation and toxicity; however, vinyl chloride as a liquid in culture medium was ineffective to suggest that cells were exposed to doses too low to produce an effect by this approach. Although trichloroethylene doses high enough to produce toxicity did not induce transformation, it is possible that exposure to trichloroethylene as a vapor could have been a stronger test as shown for vinyl chloride.

The study by Purchase et al. (1978), which was done on 120 chemicals of various classes, showed that the BHK-agar transformation assay system was about 90% accurate in discriminating between compounds with demonstrated



— Survival and transformation of BHK cells treated with vinyl chloride gas (●——●) and vinyl chloride solution in DMSO (▲— . —▲).

Survival and transformation of BHK cells treated with unstabilized trichloroethylene solution in DMSO

Figure 2. Cell transformation assays on vinyl chloride and trichloroethylene in BHK-21/Cl 13 _in vitro_. (Styles 1977, 1979)

carcinogenic or noncarcinogenic activity and was in approximately 83% agreement with results from assays done by the authors with S. typhimurium (TA 1535, TA 1538, TA 98, TA 100). Styles (1979) and Purchase et al. (1978) indicated, without presenting numerical data, that similar results were obtained in the Salmonella assays on vinyl chloride liquid and vapor and trichloroethylene liquid was similar to results found in the transformation assays. Purchase et al. (1978) also observed that metabolically activated agents transformed BHK cells more strongly in the presence of S-9 mix, thus suggesting that BHK cells have limited intrinsic metabolic capability.

V. TOXICITY

Humans are exposed to trichloroethylene primarily as a vapor. Indications of trichloroethylene toxicity include central nervous system depression, manifested, for example, by eyesight disturbances; mental confusion; fatigue; nausea and vomiting; irregular heartbeat; irregular breathing; irritation of the lung, gastrointestinal tract, and skin; and disturbances in protein metabolism (IARC 1976, NCI 1976). Kleinfeld and Tabershaw (1954) concluded that deaths from exposure to trichloroethylene occur mainly from ventricular fibrillation, possibly attributable to increased sensitization of the heart to catecholamines (Cornish 1975). Trichloroethylene residue levels of 1 to 32 ug/kg wet tissues have been found in postmorten humans with a history of exposure to trichloroethylene (McConnell et al. 1975).

The approximate oral LD_{50} of trichloroethylene in mice, dogs, and rats is 3.2, 2.8, and 4.9 g/kg, respectively (Klaasen and Plaa 1966, 1967; Smyth et al. 1969), and an approximate inhalation LC_{50} of trichloroethylene of 0.22 ± 0.01 g/l air (40,260 ppm), 20 minutes exposure, in mice (Aviado et al. 1976), and 0.067 g/l air (12,500 ppm), 4 hours exposure, in rats (Siegel et al. 1971), has been reported.

Exposure to trichloroethylene vapor 7 hours daily, 5 days/week, for 6 months did not produce toxic effects at concentrations of 200 ppm (1.09 mg/l air) in rats and rabbits, 100 ppm (0.55 mg/l air) in guinea pigs, and 400 ppm (2.18 mg/l air) in monkeys examined for growth, hematology, behavior, clinical chemistry, and pathology; however, exposure to 400 ppm increased liver and kidney weights in rats and liver weights in rabbits and decreased body weight (also produced at 200 ppm) in guinea pigs (Adams et al. 1951). The authors reported that, although the impurities were not identified, the trichloroethylene sample was

98.0 to 99.7% pure. Prendergast et al. (1967) did not observe treatment-related effects with regard to growth, behavior, hematology, clinical and histo-chemistry, and pathology of trichloroethylene vapor in rats, guinea pigs, monkeys, rabbits, and dogs exposed either to 3,825 mg/m^3 air (700 ppm), 8 hours/day, 5 days/week, for 6 weeks or to 189 mg/m^3 air (34.6 ppm) continuously for 90 days with the exception of slight body weight loss in dogs under repeated exposure and rabbits under continuous exposure. The trichloroethylene used was described as being the highest reagent grade purity available with a boiling point range of 86.6 to 86.9°C. Centrilobular necrosis, nephritis, hypertrophy of the lymphoid glands, and splenomegaly were detected in cats exposed to 20 ppm (0.11 mg/l air) trichloroethylene vapor 1.0 to 1.5 hours/day for 4 to 6 months (Mosinger and Fiorentini 1955). The composition of the test material was not described. Mazza and Brancaccio (1967) concluded that treatment of 12 rabbits with 2,790 ppm (15 mg/l air) trichloroethylene vapor 4 hours daily, 6 days/week, for 45 days resulted in myelotoxic anemia caused by a direct action of trichloroethylene on bone marrow. Findings reported by these authors included normochromic anemia, leukopenia, thrombocytopenia, and myelograms showing hypoplasia of erythroblastic parenchyma and an increase of lymphatic and reticuloendothelial cells. The composition of the trichloroethylene test sample was not described by the authors.

Tucker et al. (1980) evaluated the toxicity of trichloroethylene (certified ACS, 99.9% pure, $\leq$ 0.05% six unidentified impurities, 0.004% diisopropylamine stabilizer) in subchronic 4- and 6-month studies in which groups of male and female CD-1 mice given 0.1, 1.0, 2.5, or 5.0 mg/ml of trichloroethylene in 1% emulphor in deionized drinking water were compared with untreated and vehicle-treated controls. Time-weighted average daily dosages were 19, 220, 395, and 660 mg/kg in males and 18, 200, 450, and 800 mg/kg in females; fresh trichloroethylene solutions were presented twice weekly. Animals were examined with respect to growth, pathology, water consumption, hematology, urinalysis, clinical chemistry, and hepatic microsomal enzyme activities. Pathologic changes were not observed in the lowest dosage group. Pathologic abnormalities were found in the three highest dosage groups and included: livers described as pale, granular, having white spots, and having a nodule (one high-dose male); gas pockets and blood in the intestines; and hyperemia in the lungs. Pale, blotched livers were found in both control groups. Statistically significant ($P < 0.05$) differences between vehicle control and treatment groups were calculated; however, since the study report is currently in a prepublication stage, a qualitative description of these differences is presented herein. Although a clear dose-response relationship is not apparent, differences found between vehicle control and treatment groups include the following: decreased body weights in high dose males at 4 and 6 months; increased liver weights and/or body weight ratios in males at 4 and 6 months and females at 4 months in the three highest dose groups; increased kidney/body weight ratios in high dose males at 6 months and high dose females at 4 and 6 months; decreased leukocyte counts in females in the three highest dose groups at 4 months; increased fibrinogen levels in males in the three highest dose groups at 4 and 6 months; decreased prothrombin times in all dose groups of females at 6 months. Water consumption was lower in males in the two highest dosage groups compared to the vehicle control.

Sanders et al. (1980) estimated the effect of trichloroethylene on humoral and cell-mediated immunologic responses in mice on study in the evaluation by Tucker et al. (1980). Humoral immunity, characterized as the primary I_gM response to sheep erythrocytes, was determined by a hemolytic plaque assay performed with animals immunized 4 to 5 days before sacrifice with intraperitoneal injections of 4 x 10^8 sheep erythrocytes; plasma antibody titer was also measured by the hemagglutination methods in plasma obtained 7 days after intraperitoneal injection of 1 x 10^9 sheep erythrocytes. Cell-mediated immunity was examined by sensitizing the animals with an injection of 1 x 10^8 erythrocytes into the left footpad followed 4 days later with intravenous administration of 24,000 cpm ^{125}I-human serum albumin. Two hours after treatment with ^{125}I, the mice were sacrificed, and both hind feet were removed for counting radioactivity. Cell-mediated immunity was expressed as a stimulation index calculated as follows: (cpm left footpad sensitized - cpm left footpad unsensitized) ÷ (cpm right footpad sensitized - cpm right footpad unsensitized). Lymphocyte responsiveness to the B-cell mitogen LPS (lipopolysaccharide from Salmonella typhosa 0901) and the T-cell mitogen Conconavalin A was determined by incubating a suspension of spleen cells with one of the mitogens at 37°C for 48 hours with an additional incubation for 18 hours in the presence of ^{125}I-iododeoxyuridine/fluorodeoxyuridine (IUDR/FUDR) solution for counting of radioactivity. Bone marrow deoxyribonucleic acid synthesis was assessed by incubating marrow cells from the femur with ^{125}I-IUDR/FUDR solution and by counting radioactivity in the acid precipitable fraction in incubate samples removed at 60, 120, and 180 minutes; additionally, density of the bone marrow cells population was estimated by counting colonies on plates containing cell suspension incubated at 37°C for 7 days. Several tests were done to evaluate macrophage function. Cells collected from the peritoneum after intraperitoneal injection of 10% Brewer's thioglycollate were counted on a Coulter counter, and cell adherence was measured by incubating plates containing 2 x 10^5 cells/plate for 18 to 24 hours at 37°C and then counting cells remaining on each plate after extensive washing. Phagocytosis was studied by incubating 2 x 10^5 cells with opsonized ^{51}Cr-labeled sheep erythrocytes in 24 well dishes for 10 to 45 minutes, followed by counting for radioactivity. The cells were exposed to hypotonic shock prior to counting to differentiate phagocytosis from erythrocyte adherence. Chemotaxis of the cells was assessed by placing 1 to 2 x 10^6 cells/ml and vehicle or chemoattractant into blind well Boyden chambers containing 13 mm membrane filters for a 4-hour incubation at 37°C. Subsequently, the cells were removed for histologic fixation and staining for counting in microgrid fields.

Statistically significant ($P < 0.05$) differences were found between vehicle control and treatment groups; however, since the study report is presently in a prepublication stage, a qualitative description of the results is presented below. The humoral immune response, as indicated by decreased levels of antibody-forming cells and hemagglutination titers, in the two highest dose groups and the decreased cell-mediated immune response in all dose groups were apparent in females after 4 months of exposure but were not evident in females treated for 6 months. Although a reduction in the number of antibody-forming cells per spleen was found in males treated for 6 months with the two highest dose levels, the immune system in males was relatively unaffected compared to females from similar treatment with trichloroethylene in drinking water. An effect of trichloroethylene on specific macrophage functions was not obvious in this test. A reduction in humoral and cell-mediated immunologic responses in 28-day-old CD-1 mice dosed with either 24 or 240 mg trichloroethylene/kg by gavage daily for 14 days was also demonstrated by Sanders et al. (1980). In the humoral test, mice were sensitized to sheep erythrocytes on day 10 and sacrificed on day 15, and in the cell-mediated test mice were sensitized to sheep erythrocytes on day 10 and challenged on day 14 before sacrifice on day 15. The humoral response was determined as the number of antibody-forming cells per spleen and per 10^6 spleen cells, and the cell-mediated response was estimated by the stimulation index previously described herein. Statistical analysis of the results showed a significant ($P < 0.05$) dose-related decrease in the cell-mediated response in both treatment groups. A statistically significant effect on the humoral response was not discerned; however, a dose-related decrease in this response in both treatment groups was also evident.

Sanders et al. (1980) concluded that the immune system in CD-1 mice appears to be a target organ for trichloroethylene toxicity; however, these authors also stated that the effect of subchronic exposure to trichloroethylene in drinking water on the immune system was not remarkable in their study. At the request of the Carcinogen Assessment Group, T. Borsos, Sc.D., Chief of the Humoral Immunity Section at the National Cancer Institute, has kindly provided a written evaluation (1981) on the immunotoxicology study by Sanders et al. (1980). The opinion expressed by Dr. Borsos in reference to the drinking water study is that, if trichloroethylene had an effect on the immunologic response measured in this study, it was so small that it was obscured by variations found in the data for naive and vehicle control animals.

The conclusions by Sanders et al. (1980) indicate that the results of the immunologic tests by Sanders et al. (1980) may provide supporting evidence with respect to the conclusion by Henschler et al. (1980) that the increased incidence of malignant lymphoma in treated female mice in their carcinogenicity study of trichloroethylene possibly could have been related to an occurrence of immunosuppressive in these animals. This issue is further discussed on pages 100 and 102.

VI. CARCINOGENICITY

ANIMAL DATA

National Cancer Institute Study

A carcinogenicity study of trichloroethylene in B6C3F1 mice and Osborne-Mendel rats was reported by the National Cancer Institute (NCI) in 1976. The composition of the trichloroethylene sample used, determined by vapor-phase chromatography-mass spectrometry, was as follows: trichloroethylene, $\geq$ 99.0% (w/w); 1,2-epoxybutane, 0.19%; ethyl acetate, 0.04%; N-methylpyrrole, 0.02%; diisobutylene, 0.03%; epichlorohydrin, 0.09%. Treatment groups included 50 animals/sex/dosage, and 20 animals/sex comprised matched vehicle control (corn oil) groups. Ninety-nine male and 98 female rats and 77 male and 80 female mice used as vehicle "colony controls" and 70 male and 76 female mice serving as untreated "colony controls" were also compared with treatment groups in this study; vehicle "colony controls" included matched controls for trichloroethylene and other chemicals being tested concurrently.

Time-weighted average dosages of trichloroethylene in mice were 1,169 and 2,339 mg/kg/dose in males and 869 and 1,739 mg/kg/dose in females. Dosing was done by gavage 5 days/week, for 78 weeks, and the animals were sacrificed for pathologic examination at 12 weeks following treatment. Both male and female rats were given time-weighted average dosages by gavage of 549 and 1,097 mg trichloroethylene/kg/dose, 5 days/week, for 78 weeks and were subjected to pathologic examination on sacrifice at 32 weeks post-treatment. The selected dosages were those determined to be maximally tolerated, i.e., the highest dosage estimated from a preliminary 8-week subchronic test which would not be expected to alter survival rate from effects other than carcinogenicity during the chronic study, and one-half maximally tolerated. In order to maintain this dosage regimen, dosages were changed at 7 and 16 weeks for rats and 12 weeks for mice after the study began. Furthermore, the dosing schedule for rats was changed to a cycle of 1 week without treatment followed by 4 weeks of treatment during the final 48 weeks of dosing in an attempt to improve survival. Positive control groups (50 animals/sex/dosage) were treated with carbon tetrachloride for 78 weeks, 5 days/week, followed by nontreatment for 12 (mice) and 32 (rats) weeks until sacrifice; time-weighted average doses (mg/kg/dose) were 47 and 94 for male rats, 80 and 159 for female rats, and 1,250 and 2,500 for male and female mice.

Peak average body weights in male rats were 622 g at 46 weeks in matched controls, 575 g at 46 weeks with the low dosage, and 535 g at 38 weeks with the high dosage, and in female rats were 404 g at 62 weeks in matched controls, 322 g at 70 weeks with the low dosage, and 326 g at 94 weeks with the high dosage. Body weights subsequently decreased in all groups until the end of the study. Survival of treated rats was poor and was attributed to intercurrent disease and/or an appreciable sensitivity to systemic toxic effects of trichloroethylene, although the only treatment-related lesion was reported to be chronic nephropathy. Median survival times were as follows: 88 weeks for matched control males, 85 weeks for low dosage males, 62 weeks for high dosage males, 103 for matched control females, 74 weeks for low dosage females, and 68 weeks for high dosage females. A high incidence of chronic respiratory disease was determined in all groups.

A carcinogenic effect of trichloroethylene was not discerned in rats; however, the results may be inconclusive with respect to the high mortality previously described. Total tumor incidences expressed as the number of rats with tumors were as follows: 5/20 matched controls, 7/50 low dosage, and 5/50 high dosage for males, and 7/20 matched controls, 2/48 low dosage, and 12/50 high dosage for females.

Average body weights peaked at 34 g and 27 g in male and female mice, respectively, in all groups at 34 weeks, and these body weights were maintained until the end of the experiment. The median survival times were as follows: 77 weeks for matched control males, > 90 weeks for low dosage males, 77 weeks for high dosage males, and > 90 weeks for females in the matched control and both treatment groups. Prior to sacrifice, approximately 50% of the treated males and few treated females exhibited bloating or abdominal distension.

A treatment-related statistically significant ($P < 0.05$) increase in the incidence of hepatocellular carcinomas in male and female mice treated with trichloroethylene was determined (Table 16). Metastasis of this carcinoma to the lung was observed at an incidence of 8% in treated males, whereas metatases are very rare in controls. The carcinogenic response in females was clearly related to dosage. Although not statistically significant, the incidence of malignant lymphoid tumors (reticulum cell sarcoma and lymphosarcoma) was slightly higher in the treated animals as follows: 1/20, matched control males; 4/47, low dosage males; 2/45, high dosage males; 1/19, matched control females; 5/48, low dosage females; and 6/47, high dosage females. With respect to respiratory system tumors, benign pulmonary adenomas in 5/50 low dosage males, 1/48 high dosage males, 1/20 matched control females, 3/50 low dosage females, and 5/47 high dosage females as well as alveolar adenocarcinomas in 1/48 high dosage males (with metastasis), 2/49 low dosage females, and 2/47 high dosage females were found; however, the differences between matched control and treatment animals are not statistically significant.

In mice, hepatocellular carcinomas were found in 49/49 low dosage males, 47/48 high dosage males, 40/40 low dosage females, and 43/45 high dosage females treated with carbon tetrachloride. With regard to "colony control" mice,

TABLE 16. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN B6C3F1 MICE RECEIVING TRICHLOROETHYLENE BY ORAL GAVAGE (NCI 1976)

	Males			Females		
Dose	0 mg/kg	1169 mg/kg	2339 mg/kg	0 mg/kg	869 mg/kg	1739 mg/kg
Number with tumors						
Number of survivors	1/20	26/50	31/48	0/20	4/50	11/47
Incidence	0.05	0.52	0.646	0	0.08	0.234
P-value		1.39×10^{-4}	3.44×10^{-6}		1.75×10^{-1}	1.35×10^{-2}
Number with metastases	0/20	4/50	3/48	0/20	0/50	0/47

hepatocellular carcinomas were diagnosed in 5/77 (6.5%) vehicle control and 5/70 (7.1%) untreated control males and 1/80 (1.3%) vehicle control and 2/76 (2.6%) untreatment control females. Hepatocellular carcinoma incidences in "colony control" mice were comparable to those in matched control males (1/20 or 5%) and matched control females (0/20 or 0%). In contrast, hepatocellular carcinoma incidences in rats given carbon tetrachloride were 2/50 in low dosage males, 2/50 in high dosage males, 4/49 in low dosage females, and 1/49 in high dosage females. In rats dosed with carbon tetrachloride, marked hepatotoxicity with fibrosis, bile duct proliferation, and regeneration was observed, and, except for hepatic neoplastic nodules diagnosed in 2/50 low dosage males, 1/50 high dosage males, 2/49 low dosage females, and 3/49 high dosage females, hepatic nodules in rats treated with carbon tetrachloride were classified as regenerative. Hepatocellular carcinoma was found in 1/99 "colony control" male rats, and hepatic neoplastic nodules were observed for 2/99 "colony control" female rats. However, carcinoma and neoplastic nodules were not found in livers from 20 matched control male and 20 matched control female rats. By the Fisher Exact Test, statistically significant ($P < 0.05$) differences in liver tumor incidence between matched control rats and treated rats were not found. Statistical comparison with "colony control" animals revealed a significant increase in the combined incidence of carcinoma and neoplastic nodules in livers from low dosage male rats (1/99 vs. 4/50, $P = 0.044$) and low dosage female rats (2/98 vs. 6/49, $P = 0.017$) as well as in the incidence of carcinomas alone in livers from low dosage female rats (0/98 vs. 4/49, $P = 0.011$); however, differences in liver tumor incidence between "colony control" rats and high dosage rats were not statistically significant. The 78-week survival incidences of rats treated with carbon tetrachloride were 34/50 or 38/49 males and females in each treatment group with the exception of 21/49 high dosage females.

Sixty-seven male and 75 female "colony control" rats survived for 78 weeks. Although the rats were treated with carbon tetrachloride dosages considerably lower than those used to treat the mice, both species were treated with maximally tolerated dosages of carbon tetrachloride in this study. Consequently, there appears to be a difference in the sensitivity of the Osborne-Mendel rats and B6C3F1 mice under the experimental conditions of this study to the induction of hepatocellular carcinoma by carbon tetrachloride.

Industrial Bio-Test Laboratories, Inc. Study

A carcinogenicity study of trichloroethylene vapor in B6C3F1 mice and Charles River rats was conducted at Industrial Bio-Test Laboratories, Inc. (IBT) from 1975 to 1977, and audit findings by the Manufacturing Chemists Association (MCA) on this study have been reported (Bell et al. 1978). The trichloroethylene product used in this study contained, on a weight percent basis, trichloroethylene, > 99%; diisobutylene, 0.023%; butylene oxide, 0.245%; ethyl acetate, 0.052%; N-methylpyrrole, 0.008%; and epichlorohydrin, 0.148%. Each of the three treatment groups plus an untreated control group consisted of 140 mice/sex and 120 rats/sex. Treated animals were exposed to nominal concentrations of 100, 300, or 600 ppm (0.55, 0.165, or 0.330 mg/l air) trichloroethylene vapor 6 hours/day, 5 days/week, for 24 months. Surviving animals were sacrificed on termination of treatment. Forty mice/sex and 30 rats/sex scheduled for interim sacrifice to evaluate hematology and clinical chemistry were not given a pathologic examination.

According to the audit report by MCA, deficiencies in the performance of this study occurred which significantly compromise the interpretation of the data. Although all mice evidently were received from the same supplier, control mice appear to have been selected from a shipment obtained approximately 3 weeks after the shipment used as the source for treatment groups of mice. Moreover, examination of analytical data on the trichloroethylene vapor concentrations in the inhalation chambers revealed wide deviations from the nominal concentrations, and high variability in the number of measurements made daily which do not allow a precise determination of the actual exposure levels. For example, histograms of measurements (Figures 3, 4, and 5) made during the initial 120 days of exposure show a clustering of data around the nominal concentrations; however, only 259/565, 393/623, and 389/598 readings made during the first 6 months of the study fell within ± 30% of the 100, 300, and 600 ppm nominal concentrations, respectively. Exposure data in the report by Bell et al. (1978) cover the initial 6 months of this study, and additional information from the Chemical Manufacturers Association has been requested regarding exposure levels obtained during the final 18 months of this study.

Nonetheless, although treatment animals could not be compared with matched controls simultaneously put on study, re-evaluation of livers histologically showed a statistically significant ($P < 0.05$) increase in the incidence of hepatocellular carcinomas in male mice and the combined incidence of hepatocellular carcinomas and adenomas in male and female mice exposed to trichloroethylene (Table 17). Furthermore, with respect to the treatment groups which were on study concurrently, an increased incidence of liver tumors in high-dose animals compared to low- and mid-dose animals is evident which may be related to the patterns of exposure level frequencies around nominal concentrations in Figures 2, 3, and 4. Statistical analysis of data in Table 8 by the Fisher Exact Test revealed a significant ($P < 0.05$) increase in hepatocellular carcinomas in males ($P < 0.024$) and females ($P < 0.019$) and hepatocellular carcinomas and adenomas combined in males ($P = 0.010$) of the high dose group compared to the low dose group. Although not significant ($P = 0.066$), the combined incidence of hepatocellular carcinomas and adenomas was

FIGURE 3. TRICHLOROETHYLENE HISTOGRAM OF CHAMBER CONCENTRATION MEASUREMENTS
T-1 (100 ppm)
(Bell et al. 1978)

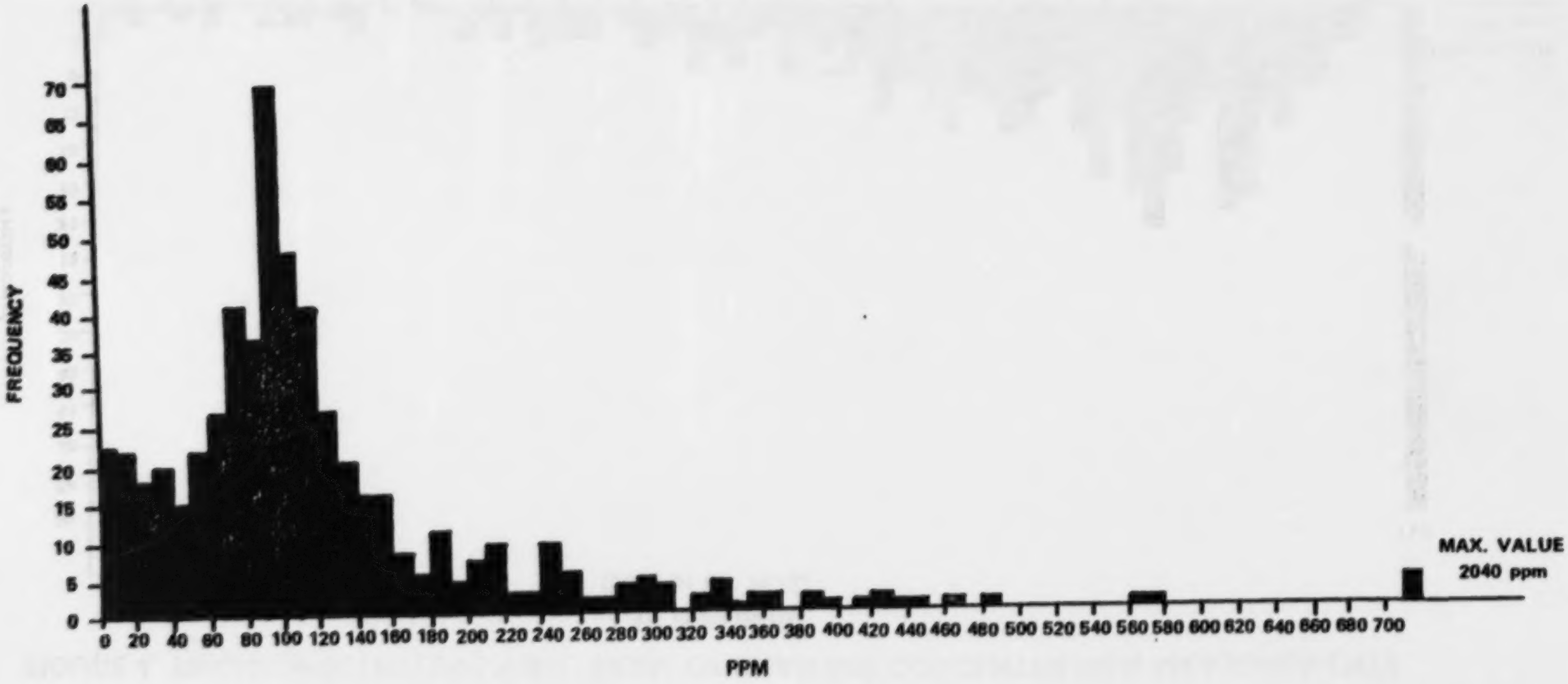


FIGURE 4. TRICHLOROETHYLENE HISTOGRAM OF CHAMBER CONCENTRATION MEASUREMENTS

T-III (600 ppm)

(Bell et al. 1978)

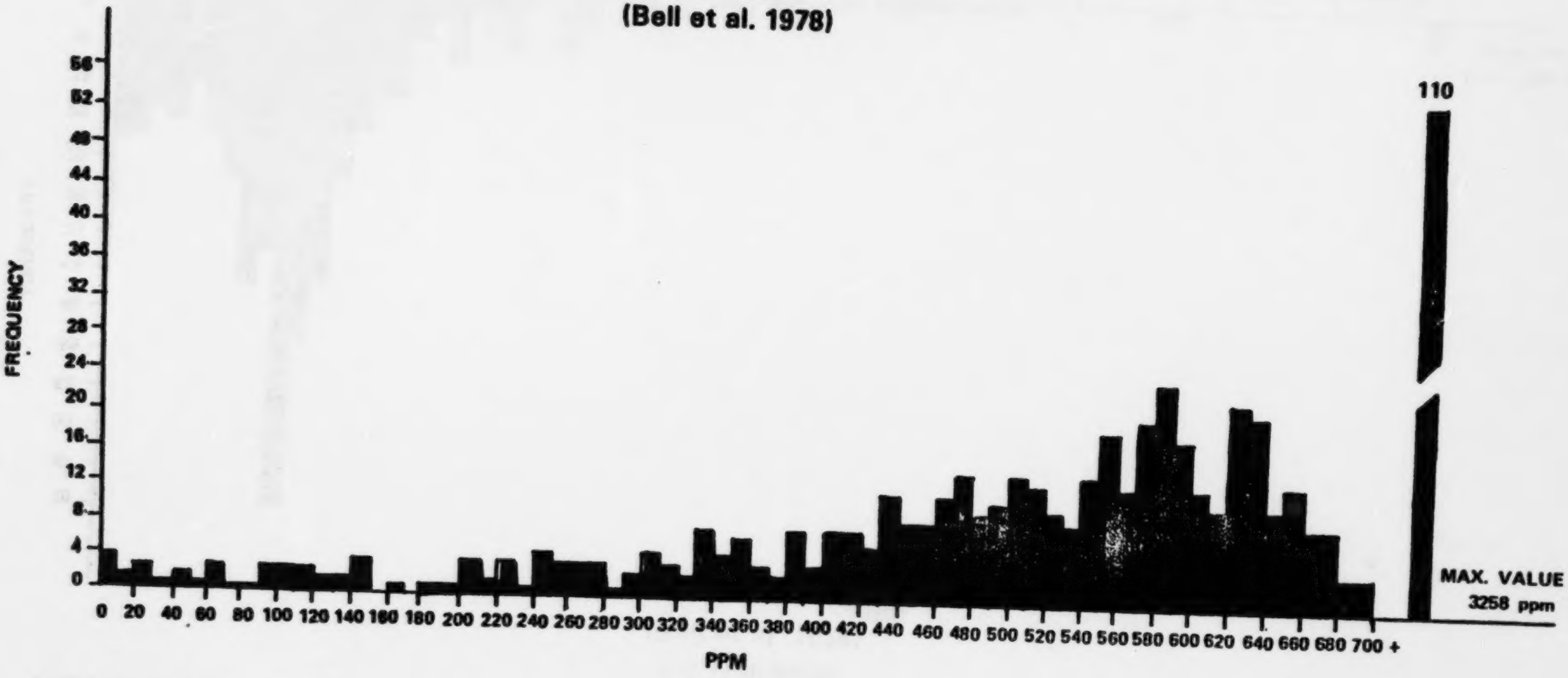


FIGURE 5. TRICHLOROETHYLENE HISTOGRAM OF CHAMBER CONCENTRATION MEASUREMENTS
T-II (300 ppm)
(Bell et al. 1978)

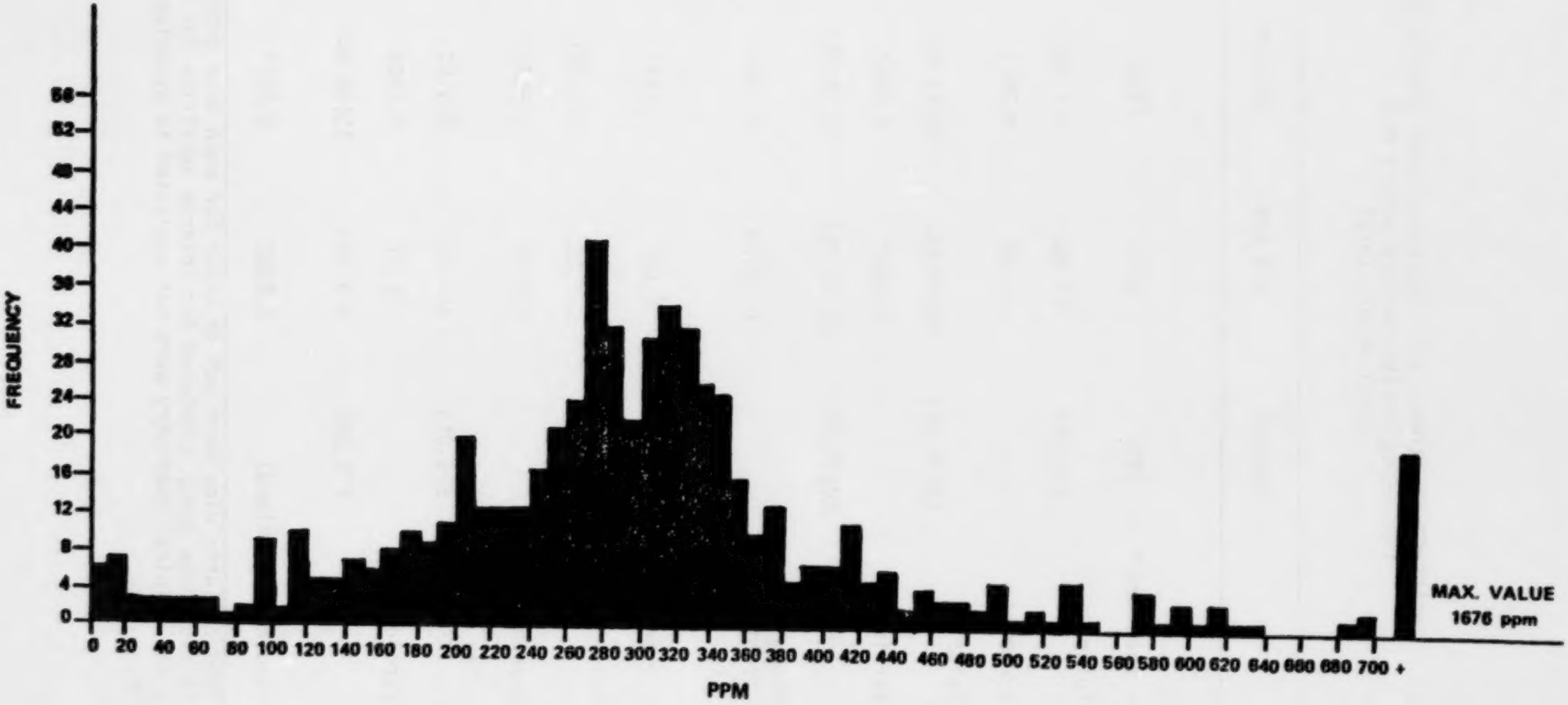


TABLE 17. INCIDENCE OF PRIMARY HEPATOCELLULAR NEOPLASMS IN CONTROL AND TRICHLOROETHYLENE-EXPOSED B6C3F1 MICE
(Bell et al. 1978)

	Control	100 ppm	300 ppm	600 ppm
Males				
Liver (no. examined)*	(99)	(95)	(100)	(97)
Hepatocellular adenoma	2(2.0%)	7(7.4%)	7(7.0%)	10(10.3%)
P-value (adenoma)		0.0752	0.0873	0.0150
Hepatocellular carcinoma	18(18.2%)	28(29.5%)	31(31.0%)	43(44.3%)
P-value (carcinoma)		0.0463	0.0262	0.000063
Total	20(20.29%)	35(36.8%)	38(38.0%)	53(54.6%)
P-value(adenoma/ carcinoma combined)		0.00778	0.00441	0.00000049
Females				
Liver (no. examined)*	(99)	(100)	(94)	(99)
Hepatocellular adenoma	2(2.0%)	5(5.0%)	1(1.0%)	4(4.0%)
P-value (adenoma)		0.2265	0.8672	0.3413
Hepatocellular carcinoma	6(6.0%)	4(4.0%)	9(9.6%)	13(13.1%)
P-value (carcinoma)		0.8382	0.2604	0.0730
Total	8(8.0%)	9(9.0%)	10(10.6%)	17(17.1%)
P-value (adenoma/carcinoma combined)		0.5083	0.3579	0.0427

---

*One hundred-forty mice were put on study for each dose group; however, 40 mice/sex in each dose group scheduled for interim sacrifice for evaluation of hematology and clinical chemistry were not subjected to pathologic examination.

higher in high dose females compared to low dose females. Histologic re-examination of livers from rats did not show a carcinogenic effect from exposure to trichloroethylene. Hepatocellular carcinomas were found in 3/99 control male and 3/99 low-dose male rats.

CONTAMINANTS OF TECHNICAL TRICHLOROETHYLENE

Epichlorohydrin and 1,2-epoxybutane, both monofunctional, alkylating agents are known mutagens (Fishbein 1979), and epichlorohydrin has been demonstrated to be carcinogenic. In female ICR/Ha Swiss mice, epichlorohydrin was shown to be a tumor initiator localized at the site of treatment when applied to the skin at a single dose of 2 mg followed by dermal application of 2.5 ug phorbol myristate acetate three times weekly for the remainder of the 385-day experiment and when injected subcutaneously at a weekly 1 mg dose for 580 days. Repeated application of epichlorohydrin itself onto the skin was ineffective. Induction of hepatic carcinomas by epichlorohydrin was not found in this study (Van Duuren et al. 1967). Laskin et al. (1980) found induction of squamous cell nasal carcinoma formation in 15 of 140 rats exposed to 100 ppm (0.384 mg/l air) epichlorohydrin by inhalation 6 hours/day, 5 days/week, for 30 exposures with subsequent lifetime observation compared to zero incidence of this cancer type in 50 untreated matched controls and several thousand historical controls. Van Duuren et al. (1967) found that 1,2-epoxybutane was not carcinogenic in female ICR/Ha Swiss mice when applied to the skin (100 mg of a 10% solution) three times weekly in a 540-day experiment.

Consequently, there is a possibility that epichlorohydrin and 1,2-epoxybutane could have contributed to the carcinogenic properties of the trichloroethylene samples used in the NCI and IBT studies. However, carcinogenicity studies on epichlorohydrin and 1,2-epoxybutane in mice comparable to those reported by the NCI and IBT have not been done to the knowledge of the CAG. Hence, under the experimental conditions of the NCI and IBT studies, a carcinogenic effect of trichloroethylene itself in mice cannot be decided because of the possible effects of the contaminants. One line of evidence suggests that the contamination by epichlorohydrin and 1,2-epoxybutane did not contribute significantly to the the mouse hepatoma response in the NCI bioassay of technical trichloroethylene. The Van Duuren study in which a 1 mg subcutaneous dose of epichlorohydrin was given once a week for 83 weeks showed no hepatoma response in female mice (Van Duuren et al. 1967). The high dose of 1739 mg/kg/day in female mice in the NCI study showed a positive hepatoma response with an equivalent epichlorohydrin dose of 8.5 mg/kg in contrast to the negative Van Duuren study with a higher weekly dose of 33 mg/kg of epichlorohydrin. In this comparison, the combined 1,2-epoxybutane and epichlorohydrin dose in the positive NCI bioassay was about equal to that for epichlorohydrin alone in the negative Van Duuren study. It is not known whether 1,2-epoxybutane is carcinogenic at all but, if so, it is unlikely to be stronger than epichlorohydrin since both tested negative as whole skin carcinogens in the mouse. However, this above line of evidence is only suggestive that the positive NCI bioassay was not due to contaminant epoxides since there are additional uncertainties due to the lack of comparability between the Van Duuren and NCI studies with respect to routes of administration patterns (5 days/week by gavage versus 1 day/week by subcutaneous injection) and mouse strain (ICR/Ha Swiss versus B6C3F1). Additional carcinogenicity studies on trichloroethylene, free of detectable epoxides, besides that completed by Henschler et al. (1980) and reviewed below, are ongoing as described herein. As these additional studies become available for review, the weight of evidence with respect to the carcinogenicity of trichloroethylene can be re-evaluated.

Henschler et al. Study

Henschler et al. (1980) recently reported a study on the carcinogenic potential of trichloroethylene in Han:NMRI mice, Han:Wist rats, and Syrian hamsters. Thirty males and 30 females of each species were assigned to each of three dose groups which were exposed to 0 (untreated controls), 100 (0.55 mg/l air), or 500 (2.8 mg/l air) ppm trichloroethylene vapor 6 hours daily, 5 days/week, for 18 months. The animals were individually caged in this study. Surviving mice and hamsters were sacrificed at 30 months, and surviving rats were sacrificed at 36 months. All animals were subjected to necropsy and histopathologic examination.

The trichloroethylene sample, stabilized with 0.0015% triethanolamine, contained the following impurities by gas chromatography-mass spectrometry analysis: chloroform, carbon tetrachloride, 1,1,2-trichloroethane, 1,1,1-trichloroethane, and 1,2,2-tetrachloroethane, each present at less than 0.0000025% (w/w). Epoxides were not detected in this test material. Exposure levels of trichloroethylene in the inhalation chambers were monitored with direct ultraviolet spectrophotometry, and the flow rate of the trichloroethylene-air mixture was adjusted to yield a threefold turnover of the total volume each hour. A description of the analytical method was provided by D. Henschler, University of Wurzburg, as follows. Exposure levels were automatically controlled to 100 or 500 ppm, as appropriate, and were continuously monitored in a gas-flow cell in an ultraviolet beam of 205 mm. The accuracy of this method was determined by the noise of the electronic equipment, and the registered band indicated ± 7% of the median at 100 ppm.

No effect of trichloroethylene on body weight gain was observed. Survival patterns described in Table 18 show a greater mortality in treated mice compared to controls. A treatment-related effect on survival of rats and hamsters is not

TABLE 18. PER CENT SURVIVAL OF ANIMALS
(adapted from Henschler et al. 1980)

	Mice Control		100 ppm		500 ppm		Rats Control		100 ppm		500 ppm		Hamsters Control		100 ppm		500 ppm	
Week	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
75	83.8	83.3	63.3	73.3	56.6	82.8	93.3	96.6	96.6	93.3	100.0	90.0	100.0	83.3	100.0	89.7	100.0	76.6
100	66.7	46.7	36.6	30.0	26.7	37.9	86.7	86.7	90.0	86.6	90.0	76.6	56.7	23.3	63.3	20.7	70.0	30.0
126	23.3	26.7	10.0	3.3	3.3	3.4	66.7	56.6	80.0	50.0	66.6	50.0	13.3	----	6.7	----	26.7	----
156	----	----	----	----	----	----	46.7	16.7	23.3	13.3	36.7	16.7	----	----	----	----	----	----

evident; however, a marked increase in the death rate for hamsters in all groups ensued after 75 weeks.

A carcinogenic effect of trichloroethylene in rats, hamsters, and male mice was not evident in this study; however, the incidence (number affected/number examined) of malignant lymphomas in female mice was as follows: 9/29 (control), 17/30 (100 ppm), 18/28 (500 ppm). The response in the low ($P = 0.042$) and high ($P = 0.012$) dose groups represents a statistically significant ($P < 0.05$) increase over the control incidence by the Fisher Exact Test. The time-to-tumor occurrence was shorter in treated females and the majority of lymphomas were apparent after treatment was terminated at 78 weeks as illustrated in Figure 6. Induction of hepatocellular carcinoma formation by exposure to trichloroethylene was not apparent in this study. Hepatocellular adenoma was diagnosed in one control and two low-dose male mice, and hepatocellular carcinoma was discerned in one male control mouse.

Use of a maximum tolerated dose is not indicated in the study report, and the animals were permitted to have a rather long recovery period after treatment. Higher exposure levels of trichloroethylene possibly could have been used to more strongly challenge the animals for carcinogenicity.

According to the authors, the increased incidence of malignant lymphoma in female mice could have been due to an occurrence of immunosuppression capable of enhancing the susceptibility to tumor induction by specific, mostly inborn, viruses. The 30% incidence of malignant lymphoma in matched control female mice in the Henschler et al. (1980) study is higher than the 12 to 22% (average 16%) range of lymphoma incidence determined by Luz (1977) between 1968 and 1977 for 830 untreated female NMRI-Neuherberg mice used as control groups in long-term experiments. Under the conditions described in the preliminary report of their study, Sanders et al. (1980) have indicated a dose-related reduction in the

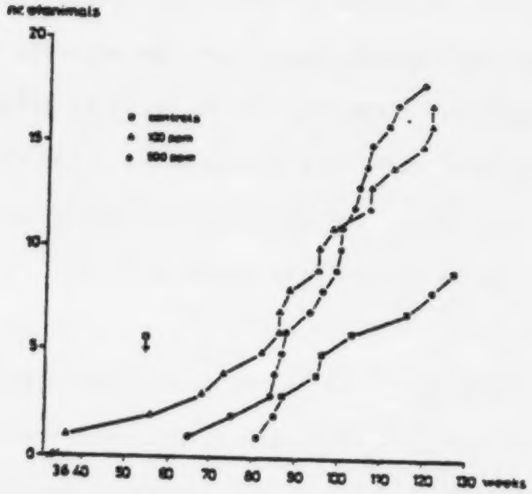


Figure 6. Incidence of malignant lymphomas in female mice.

immune response of male CD-1 mice given gavage doses of 24 or 240 mg/kg of trichloroethylene for 14 days; therefore, enhancement of the lymphoma incidence in the treated female mice possibly could have be due to an immunosuppressive action of trichloroethylene. Kreuger (1972) presented an argument similar to that of Henschler et al. (1980) in which a coexistence of persistent antigenic stimulation and chronic immunosuppression is pathogenetically related to malignancy in immunocompetent tissues. Furthermore, Kreuger (1972) indicated that a significant increase in the incidence of lymphoreticular neoplasms corresponded to a significantly shortened latent period of tumor formation in several experiments cited in his report. The argument of Henschler et al. (1980), therefore, may indeed be a valid interpretation of the results of their study.

Immunosuppression leading to an increased lymphoma incidence in female mice was proposed as a mechanism of action by Henschler et al. (1980) in their carcinogenicity study on trichloroethylene, and Sanders et al. (1980) reported a reduced immunity in mice given trichloroethylene by gavage or in drinking water. Therefore, a possibility that the increased incidence of hepatocellular tumors observed in mice treated with trichloroethylene in the NCI (1976) and IBT (Bell et al. 1978) studies was related to an immunosuppressive effect of trichloroethylene, is evident. Kroes et al. (1975) evaluated the effect of immunosuppression with a purified fraction of antilymphocytic serum on liver tumor induction by N-hydroxy-N-2-fluorenylacetamide in male Fischer 344 rats, and Frankel et al. (1970) investigated the possible correlation between immunosuppression by azathioprine and liver tumor induction by N-hydroxy-N-2-fluorenylacetamide in male and female Fischer 344 rats. The effect of the liver carcinogen was not concluded to be potentiated by treatment with immunosuppressant in these studies. Results of the studies by Kroes et al.

(1975) and Frankel et al. (1970) suggest that the enhanced hepatocellular tumor formation in treated mice in the NCI and IBT studies was not attributable significantly, if at all, to an immunosuppressive effect of trichloroethylene.

Maltoni Study

Maltoni (1979) summarized the results of a study done to estimate the carcinogenic potential of trichloroethylene in Sprague-Dawley rats. Thirty males and 30 females, 13 weeks old, were assigned to each group. Two treatment groups received 50 or 250 mg/kg of trichloroethylene by gavage daily, 4 to 5 days weekly for 52 weeks, and a vehicle-control group was given olive oil. The trichloroethylene sample was at least 99.9% pure and was contaminated with ≤ 50 ppm each of 1,2-dichloroethylene, chloroform, carbon tetrachloride, and 1,1,2-trichloroethane. No detectable epoxides were found in this test material, and 10 ppm diisopropylamine was added as a stabilizer. Surviving animals were sacrificed at 140 weeks. All animals were given a necropsy examination, and tissues and organs were evaluated microscopically.

A carcinogenic effect was not demonstrated in this study. However, it cannot be determined whether a maximum tolerated dosage was used, and although the experiment lasted 140 weeks, the animals were dosed for only 52 weeks. Mortality patterns and toxic signs, if any, are not described to indicate the sensitivity of the animals to the trichloroethylene treatment used. Since the rats were not put on study until 13 weeks of age, the carcinogenic assessment of trichloroethylene does not incorporate the possible effect of exposure during the early phase of growth and development for these animals.

Van Duuren et al. Study

A carcinogenicity study of purified trichloroethylene in ICR/Ha Swiss mice was described by Van Duuren et al. (1979). Experimental details provided below include those obtained through written communication with B.L. Van Duuren, New York University. The trichloroethylene sample was > 99% pure and, although contaminants were not identified, epoxides were considered not to be present in the test material. It was stated that although range-finding studies were done to estimate maximum tolerated doses (doses which did not affect body weight gain or induce clinical signs of toxicity in a 6- to 8-week test), trichloroethylene doses used in the main topical application experiments were below those maximally tolerated. Trichloroethylene doses used in the skin treatment experiments were selected to permit a comparison with trichloroethylene epoxide, with respect to carcinogenicity, on an equimolar basis. This study incorporated the following experiments: 1) 30 females received topical applications of 1 mg trichloroethylene 3 times weekly for approximately 581 days; 2) 30 females were treated topically with a single application of 1.0 mg trichloroethylene followed 14 days later by applications of 2.5 ug phorbol myristate acetate (PMA) to the skin 3 times weekly until termination of the study at 452 days. It should be noted that 30 females similarly treated with trichloroethylene epoxide were also tested; 3) 30 females were given subcutaneous injections of 0.5 mg trichloroethylene weekly for 622 days; and 4) 30 males and 30 females were administered 0.5 mg trichloroethylene by gavage weekly for 622 days. A vehicle-control group of 30 mice and an untreated control group of 100 mice were included in each experiment. Dosing vehicles were acetone in the skin treatment tests and trioctanoin in the other tests. In the initiation-promotion experiment, 210 mice treated with PMA alone were also on test. The mice were 6-to 8-weeks old at the beginning of the study and were housed six to a cage. Test sites on the skin were shaved as necessary and were not covered; however, it was the authors impression that trichloroethylene was immediately absorbed and that evaporation from test sites was minimal. The animals were weighed monthly, and each animal was examined by necropsy. Tumors and lesions as well as skin, liver, stomach, and/or kidney were examined histologically.

Trichloroethylene was not determined to be carcinogenic in this study. In the initiation-promotion experiment, the numbers of mice with skin papillomas (squamous cell carcinomas) were: 4 (1) treated with trichloroethylene, 15 (3) treated with PMA alone, and none in the control groups. Repeated application to the skin produced lung and stomach tumors in 9 and 1 trichloroethylene-treated mice, respectively, 11 and 2 vehicle-treated controls, respectively, and 30 and 5 untreated controls, respectively. Lung tumors were diagnosed as benign papillomas, and stomach tumors were described as papillomas of the forestomach. Tumors were not found in treatment and control animals in the experiment with subcutaneous doses. In the experiment with gavage dosing, forestomach tumors were observed in one female and two male mice given trichloroethylene, no vehicle-control animals, and five female and eight male (one male had a squamous carcinoma in the forestomach) untreated control mice. Three mice given repeated applications of trichloroethylene epoxide onto the skin had skin papillomas. Median survival times approximated the number of days the animals were on test and body weights were comparable for both treatment and control groups in each experiment.

Although the results of this study were negative, use of maximum tolerated doses in the skin treatment experiments is not evident, and oral and subcutaneous doses were administered only once per week. Moreover, only liver, kidney, stomach, and/or skin were evaluated histologically.

Prepublication data from the laboratory of B.L. Van Duuren was included in the manuscript by Demopoulos et al. (1980) presented at the EPA Science Advisory Board meeting on airborne carcinogens held in Washington, D.C. on September 3-4,

1980. Two carcinogenicity studies on trichloroethylene epoxide in female ICR/Ha mice are described as follows: 1) 30 mice were treated with topical applications of 2.5 mg trichloroethylene epoxide in acetone three times weekly over a median survival period of 526 days, and 2) subcutaneous injections of 0.5 mg trichloroethylene epoxide in tricaprylin once each week into 30 mice with a median survival time of 547 days. From the information presented, it is not possible to determine whether the doses selected were maximally tolerated. Groups of 30 vehicle-control and 100 untreated controls with median survival times $\geq$ 524 days were included in these tests. Similarly maintained positive control groups of 30 animals with mean survival times of 462 and 475 days were given 3.3 mg d,1-diepoxybutane in the skin painting test and 0.33 mg d,1-diepoxybutane in the subcutaneous test.

Data on local tumors show that trichloroethylene epoxide was not carcinogenic in either test; however, data on tumor formation at sites distant from the local treatment areas, if available, were not presented to permit a fuller evaluation. In the skin painting test, local papillomas were found only in the positive control group (26 animals, 72 total papillomas). Local tumors in the subcutaneous test were described as sarcomas in one test animal, one untreated control animal, and nine positive control animals and as squamous cell carcinomas in two positive control animals.

Though done mainly to estimate general toxic effects of trichloroethylene, the studies summarized in Table 19 indicate that trichloroethylene was not carcinogenic in a variety of experimental animals. The studies involved small groups of animals and/or durations of treatment which are rather short with respect to the lifetime of the animals.

Maltoni (1979) stated that a long-term study of the toxicity of trichloroethylene given by inhalation in Sprague-Dawley rats, Swiss albino mice,

TABLE 19. SUMMARY OF NEGATIVE CARCINOGENIC DATA FOR TRICHLOROETHYLENE (Waters et al. 1977)

Species	Number	Exposure*	Results	Reference
Dogs	16	Inhalation 150-750 ppm in air 20-48 hr/wk for 7-16 wk	No tumors; no deaths	Seifter 1944
Rats Guinea pigs Monkeys Rabbits	12 11 2 4	Inhalation 3,000 ppm, 27 exposures, 36 days 100 ppm, 132 exposures, 85 days 200 ppm, 148 exposures, 212 days 200 ppm, 178 exposures, 248 days	Three rats died; no tumors	Adams et al. 1951
Cats	8	Inhalation 200 ppm, 178 exposures, 248 days	No tumors; no deaths	Mosinger and Fiorentini 1955
Mice	28	Intragastric 0.1 ml in 40% oil solution 2/wk, unspecified treatment period	No tumors; no deaths	Rudali 1967
Rats Guinea Pigs Monkeys Rabbits Dogs	15 15 3 3 2	Inhalation 3,825 mg/m^3, 8 hr/day, 5 days/wk, 6 months 189 mg/m^3 continuous for 90 days	No tumors; no deaths	Prendergast et al. 1967
Rat	Not specified	Inhalation 2,000 ppm, 8 hr/day, 5 days/wk, 3 months	No tumorigenic potential	Laib et al. 1978†

---

*Surviving animals were sacrificed on approximately the final day of exposure; however, the observation period is not specified in the report by Rudali (1967).

†Addition to summary by Waters et al. (1977).

and B6C3F1 mice is ongoing in his laboratory with completion scheduled for the end of 1981. A second carcinogenicity study of trichloroethylene given orally to B6C3F1 mice and five strains of rats (Osborne-Mendel, Fischer 344, MA540, A28807, and ACI) is being sponsored by the NCI with completion expected by December of 1981 (personal communication with T. Fischetti, NCI).

EPIDEMIOLOGICAL DATA

Axelson et al. Study

Axelson et al. (1978) did not find an excess mortality due to cancer in a cohort study of 518 male Swedish workers with a history of exposure to trichloroethylene. Workers exposed during the 50's and 60's were monitored from 1955 to 1975. Data presented in Table 20 compare observed and expected numbers of deaths in the total cohort and in subcohorts with at least 10 years latency on occupational exposure to a maximum of (low) or more than (high) an 8-hour time-weighted average of 30 ppm trichloroethylene in air. Trichloroethylene exposure was estimated from analyses of trichloroacetic acid in urine, and 100 mg trichloroacetic acid/l urine was equated with 30 ppm trichloroethylene in air. Expected numbers of deaths is the multiplication product of person-years of observation by cause- and age-specific natural death rates from respective calendar years, and fractional age- and calendar-year contributions are summarized over the entire cohort.

A carcinogenic effect of trichloroethylene in humans was not found in this study, and there was no increase in any tumor type in the cohort and subcohorts. However, possible limitations in this study include the following: 1) the actual duration of worker exposure is not known, especially since there was no guarantee that a trichloroacetic acid analysis was made each year a worker was employed; 2) although the person-year is the unit of observation, the number of workers in the cohort and subcohorts was not determined; 3) the categorization

TABLE 20. A study of mortality among men exposed to trichloroethylene in the 50's and 60's and followed 1955 through December 1975. Exposure categorization based on trichloroacetic acid (TCA) in the urine, with low exposure referring to those not exceeding a concentration of 100 mg/l TCA in urine on average.
(adapted from Axelson et al. 1978)

Cohort	Cause of Death	Person-yrs. at Observation	Observed	Expected	Risk Ratio	Confidence 95% limits Risk Ratio
Total cohort (1)	All deaths	7688	49	62.0	0.8	0.6 - 1.0
	tumors		11	14.5	0.8	0.4 - 1.4
Subcohort with $\geq$ 10 years of latency time (high and low exposure) (2+3)	All deaths	3643	37	39.8	0.9	0.7 - 1.3
	tumors		9	9.5	0.9	0.4 - 1.8
- low exp. (2)	All deaths	3095	29	32.2	0.9	0.6 - 1.3
	tumors		6	7.7	0.8	0.3 - 1.7
- high exp. (3)	All deaths	548	8	7.6	1.1	0.5 - 2.1
	tumors		3	1.8	1.7	0.3 - 4.9

of a worker into a subcohort is based on the average trichloroacetic acid level in the total number of available urinary analyses; 4) a minimum exposure period was not required for a worker to be included in the cohort, and workers beginning employment in 1965 to 1970, therefore, could have been members of the total cohort; 5) the number of workers in the subcohorts would appear to be small, particularly with regard to high exposure and > 10 years latency; and 6) the minimum latency period of 10 years required for inclusion of a worker into the subcohort might not have been sufficient for an estimate of cancer development, especially if a large portion of the subcohort barely met the 10-year requirement. Conclusions on this study take into consideration additional details provided by written communication with O. Axelson, University of Linkoping, Sweden, who also has stated that this study is being updated to include 1,100 more workers exposed to trichloroethylene during 1970 and later.

Tola et al. Study

An ongoing cohort study on workers in Finland exposed to trichloroethylene was reported by Tola et al. (1980). The cohort consisted of 1,148 men and 969 women known to have been occupationally exposed to trichloroethylene at some time between 1963 and 1976. Names of workers were obtained from the biochemical laboratory of the Institute of Occupational Health in Finland where most of the analyses for urinary trichloroacetic acid, a trichloroethylene metabolite, were done as part of the routine medical examinations for workers. Urinalyses were done by the Fujiwara reaction which is sensitive to levels as low as 1 mg trichloroacetic acid/l urine. Workers were also traced by sending name lists to workplaces and the Occupational Disease Register in Finland. After confirming worker identities vital status and cause of death were obtained from the Population Data Register of the Central Statistical Office in Finland, and cancer death information was made available by the Finnish Cancer Registry.

Urinary trichloroacetic acid levels for 2,004 workers were reported. The majority (91%) of the workers had trichloroacetic acid levels below 100 ug/l urine which, according to Axelson et al. (1978), corresponds to an ambient exposure level of 30 ppm trichloroethylene. Furthermore, 78% of the workers had urinary trichloroacetic acid levels below 50 mg/l urine. An additional 80 workers registered with clinical poisoning from exposure to trichloroethylene were also part of the cohort; however, urinary trichloroacetic acid measurements were not available for these workers.

A comparison of age-, sex-, and cause-specific mortality rates was made between the cohort and expected rates based on national mortality statistics for 1971. A subcohort of workers exposed before 1970 was also compared with the expected rates.

Although the discussion of the Tola et al. (1980) study herein focuses on carcinogenicity, it should be noted that the incidence of congenital malformations in offspring of mothers exposed to trichloroethylene was estimated. No offspring with congenital malformations were found, whereas three cases were expected.

Results on the total cohort and subcohort are summarized in Table 21. No statistically significant differences in mortality patterns and neoplasm incidences were found. Tumors were diagnosed in the gallbladder of one male, lung of three males and one female, breast of one female, uterus of three females, and testis of one male. Multiple myeloma was observed in one female. Two workers with more than 100 ug trichloroacetic acid/l urine and one worker with clinical poisoning had cancer; however, the difference from the expected number of two cases was not statistically significant.

Although an excess cancer incidence among workers exposed to

TABLE 21. COMPARISON OF THE TOTAL MORTALITY OF WORKERS EXPOSED TO TRICHLOROETHYLENE WITH THE MORTALITY OF THE TOTAL FINNISH POPULATION (adapted from Tola et al. 1980)

		Total Cohort			
		All Deaths		Neoplasms	
Age	Person-Years	Expected	Observed	Expected	Observed
		Males			
Total*	7,041	52.1	40.0	6.6	5.0
		Females			
Total*	6,892	32.2	18.0	7.7	6.0
		Subcohort (Workers Exposed Before 1970)			
		Males			
Total*	4,269	36.1	33.0	6.3	5.0
		Females			
Total*	4,822	25.8	13.0	6.0	4.0

*Only total values are presented here. In the paper by Tola et al. (1980), age-specific groups are described with the first group including ages 15 to 19, the second 20 to 24, the third 25 to 28, etc., with the last group including ages 65 and over.

trichloroethylene was not detected, the results should be interpreted with some reservation with respect to limitations in the study. Duration of follow-up was short; the age structure of the cohort was such that 60% of the males and 40% of the females were less than 40 years old at the end of follow-up; the possibility exists, as indicated by the authors, that some workers with low urinary trichloroacetic acid levels actually could have been exposed to another degreasing solvent but were included due to use of an improper exposure test by the attending physician; the actual durations of exposure were unknown. The data do indicate that trichloroethylene was not carcinogenic in humans with a short latency period of 6 to 13 years who were mainly exposed to low ambient air levels of trichloroethylene as shown by urinary trichloroacetic acid levels. The authors stated that evaluation of the cohort will continue every 5 years.

VII. UNIT RISK ESTIMATE

A unit risk estimate is calculated for technical grade trichloroethylene contaminated with epoxides based on the increased hepatocellular carcinoma incidence in treated mice in the National Cancer Institute (NCI) carcinogen bioassay. This study was selected over the other two studies showing increased tumor incidence in mice treated with trichloroethylene for several reasons. Although an oral route of administration was used, the test animals in the NCI study were dosed for the majority of their lifetimes at the maximum tolerated dosage. This compares with the inhalation carcinogenicity study by Industrial Bio-test Laboratories on technical grade trichloroethylene which suffers from wide variations in the exposure levels and the use of a control group of mice taken from a different shipment than those in the test groups. The other positive animal study is the Henschler et al. (1980) study of mice exposed to purified trichloroethylene. A unit risk estimate based on the lymphoma response in treated female mice is not made because of the possibility that the increase in lymphoma incidence was due to an immunosuppressive effect of trichloroethylene and because of the questionable nature of the dose-response relationship for immunosuppressive agents.

With regard to the effects of the epoxide contaminants in the technical grade trichloroethylene used in the NCI study, the possibility that they were unlikely to have caused the increase in hepatocellular tumors has been discussed earlier in this paper. Currently, the carcinogenicity of purified grade trichloroethylene, free of detectable epoxides, is being evaluated by the National Toxicity Program with gavage administration, and by C. Maltoni and associates with inhalation exposure in ongoing studies in various strains of rats and mice. Therefore, an estimate of unit risk from lifetime exposure to purified grade trichloroethylene is being deferred until the data from the above studies (in progress) are obtained by the Carcinogen Assessment Group (CAG) for review.

CONVERSION OF UNITS

The ppm equivalent of 1 ug/m^3 trichloroethylene is found from the following formula, where $M_{trichloroethylene}$, and M_{air} are the respective molecular weights:

$$\begin{aligned} 1 \text{ ug/m}^3 &= 10^{-3}/(1.2 \times M_{trichloroethylene}/M_{air}) \\ &= 10^{-3}/(1.2 \times 131.4/28.8) \\ &= 1.826 \times 10^{-4} \text{ ppm} \\ 1 \text{ ppm} &= 5.475 \times 10^{3} \text{ ug/m}^3 \\ 1 \text{ ppb} &= 10^{-3} \text{ ppm} = 5.475 \text{ ug/m}^3 \end{aligned}$$

Assuming that man inhales 20 m^3/day, the dosage in mg/kg/day equivalent to an exposure level of 1 ug/m^3 of trichloroethylene is

$$\begin{aligned} d &= (10^{-3} \text{ mg/m}^3 \times 20 \text{ m}^3\text{/day})/70 \text{ kg} \\ &= 0.286 \times 10^{-3} \text{ mg/kg/day} \end{aligned}$$

Data presented in the metabolism section and tabulated in Table 2 show pulmonary absorption levels of trichloroethylene in humans as high as 75% of the inhaled amount. Assuming that 75% of inhaled trichloroethylene can be absorbed into the body, 1 ug/m^3 in the air would result in an absorbed effective dose of 0.215 x 10^{-3} mg/kg/day, i.e.,

$$d = 0.286 \times 10^{-3} \text{ mg/kg/day} \times 0.75 = 0.215 \times 10^{-3} \text{ mg/kg/day}$$

For drinking water exposure it is assumed that 100% of the trichloroethylene in drinking water is absorbed and that the water intake is 2 liters per day. Under these assumptions, the daily dose from consumption of water containing 1 ug/l (1 ppb) of trichloroethylene is

$$d = 2\ \text{l/day} \times 10^{-3}\ \text{mg/l} \times 1/70\ \text{kg}$$
$$= 2.86 \times 10^{-5}\ \text{mg/kg/day}$$

NATIONAL CANCER INSTITUTE (1976) CANCER BIOASSAY IN MICE

A risk assessment is made, based on the multistage model, using the observed hepatocellular carcinoma incidence in male mice in all three dose groups of the NCI (1976) study. The linearized multistage model is used for low-dose extrapolation in order to give an estimate of a plausible upper bound of lifetime cancer risk. Using this procedure, as explained below, the carcinogenic potency factor is $q_1^* = 1.26 \times 10^{-2}\ (\text{mg/kg/day})^{-1}$.

The lifetime average exposure for the 1169 mg/kg group is

$$d = 1169 \times 5/7 \times 78/90 = 723.66\ \text{mg/kg/day}$$

Similarly, the average lifetime exposure for the high dose (2339 mg/kg) group is 1447.33 mg/kg/day.

The carcinogenic potency of trichloroethylene in mice (B_A) equals 9.90×10^{-4} as estimated by the multistage model which is described in "Methods for Determining the Unit Risk Estimate for Air Pollutants" (Carcinogen Assessment Group 1980). Tumor incidence data which provide the basis for the value are given Table 16.

Assuming that the average weight of man is 70 kg, the dose-response slope,

B_H, of the multistage curve for man is

$$B_H = A \sqrt[3]{\frac{70}{W}}$$

$$= 9.90 \times 10^{-4} \sqrt[3]{\frac{70}{0.034}}$$

$$= 1.26 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$$

where W is the weight of the animal and B_A is the slope for the animal.

Therefore, the risk for lifetime continuous exposure to 1 ug/m^3 of trichloroethylene in the air is

$$P = 1.26 \times 10^{-2} \times 0.215 \times 10^{-3}$$

$$= 2.71 \times 10^{-6}$$

The corresponding risk for drinking water containing a concentration of 1 ug/l is

$$P = 1.26 \times 10^{-2} \times 2.86 \times 10^{-5}$$

$$= 3.6 \times 10^{-7}$$

These are considered to be plausible upper bound to the estimated risks, since they are based on the upper 95% confidence limit of the linear term in the multistage model. The plausible lower bound of risk is zero, recognizing the uncertainties in both the qualitative evaluation and in the quantitative extrapolation process.

UPPER-LIMIT CALCULATION BASED ON THE AXELSON ET AL. (1978) STUDY IN HUMANS

Even though the epidemiologic studies on trichloroethylene provide no evidence for its carcinogenicity, they can still be used to provide information on an upper-limit of risk to be expected. The CAG adopts this procedure of using negative epidemiological studies for providing upper-limits when animal studies are positive. Such use of negative human studies can modify estimates of potency from the positive animal studies.

The following 95% upper-limit estimates on relative risk are derived from data in Table 20 obtained by Axelson et al. (1978). These upper-limit estimates are calculated according to the following approach.

The 95% upper-limit = u/expected number of cancer deaths (Table 22), where u is the solution to the sum of Poisson probabilities

$$\sum_{i=0}^{X} \frac{e^{-u}(u)^{i}}{i!} = 0.025$$

One might also interpret u as the upper-limit of the number of workers with cancer deaths given the observed frequency, and X = number of observed workers with cancer deaths. Hence, the 95% upper-limit estimates for the cohort and each subchort are as follows (Table 22).

TABLE 22. NUMBER OF WORKERS WITH CANCER DEATHS

Cohort	Observed X	Upper-limit u	Expected if No Effect	Relative Risk 95% Upper-limit
Total cohort	11	19.7	14.5	1.4
Subcohort with 10 yr. latency (high and low exposure)	9	17.2	9.5	1.8
Low exposure	6	13.3	7.7	1.7
High exposure	3	8.7	1.8	4.8

These upper-limit relative risks can be used to provide estimates of the upper-limit probability of death from cancer due to a lifetime exposure to 1 ug/m^3 of trichloroethylene. This upper-limit probability estimate is 1.7 x 10^5 per ug/m^3 of trichloroethylene in air and it is nearly one order of magnitude greater than the risk estimate based on the NCI (1976) carcinogenicity bioassay. In showing these risk calculations below, the CAG emphasizes that:

1. This upper-limit value is derived from a negative epidemiologic study. Not only was there no specific target organ for cancer, but the total number of cancer deaths were less than expected.
2. Since there was no specific target organ, the upper-limit lifetime probability estimate is based on all cancer deaths. Since the lifetime probability of death from cancer to humans is 0.19 (based on 1977 U.S death rates, race and sex combined), any even moderately conservative confidence limit will provide a large upper-limit risk.

The calculations are based on the subcohort data from the Axelson et al. (1978) study, specifically the low exposure group with at least a 10-year latency period. Table 22 projects an upper-limit relative risk of 1.7. Since the low exposure referred to those not exceeding a concentration of 100 mg trichloroacetic acid per ml in urine on the average, we equate this exposure to be not more than 30 ppm trichloroethylene in air. We use the 30 ppm as the 8-hour time-weighted average dose. A lower dose estimate would provide an even higher upper-limit probability of death.

For years exposed, in the absence of any other information, we chose 10 years as a point assumed to be somewhere in the middle of the range.

With the above information we estimate the total lifetime dose of trichloroethylene to the low dose exposure workers as:

$$\frac{(0\text{ ppm} + 30\text{ ppm})}{2} \times \frac{240\text{ days}}{365\text{ yrs}} \times \frac{10\text{ years}}{70\text{ lifetime}} = 1.41\text{ ppm continuous}$$

where the 30 ppm trichloroethylene of the working day must be average with the 0 ppm of the nonworking day. We assume that one-half the air breathed during a day will be during working hours. Thus, the upper-limit estimate of the probability of death from 1 ppm trichloroethylene in air (continuous exposure) is estimated as:

$$p = \frac{\alpha(R - 1)d_1}{d_2} = \frac{0.19\ (1.7 - 1)1}{1.41} = 0.094$$

where R = the relative risk, d_1 = 1 ppm, d_2 = dose of workers, and = the background lifetime probability of death from all cancers. Using the transformation 1 ppm = 5.475×10^3 ug/m^3, we calculate

$$P = \frac{0.094}{\text{ppm}} \times \frac{1\text{ ppm}}{5.475 \times 10^3\text{ ug/m}^3} = 1.7 \times 10^{-5}\text{ per ug/m}^3$$

VIII. REFERENCES

Abrahamson, S., and R. Valencia. 1980. Evaluation of substances of interest for genetic damage using Drosophila melanogaster. Final sex-linked recessive lethal test report to FDA on 13 compounds. FDA Contract No. 233-77-2119.

Adams, E.M., H.C. Spencer, V.K. Rowe, D.D. McCollister, and D.D. Irish. 1951. Vapor toxicity of trichloroethylene determined by experiments on laboratory animals. A.M.A. Arch. Indus. Hyg. Occup. Med. 4:469-481.

Ames, B.N., W.E. Durston, E.Y. Yamasaki, and E. Lee. 1973. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. (U.S.A.) 70:2281-2285.

Ames, B.N., J. McCann, and E. Yamasaki. 1975. Method for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

Astrand, I. 1975. Uptake of solvents in the blood and tissues of man. A review. Scand. J. of Work Environ. and Health 1:199-218.

Astrand, I., and F. Gamberale. 1978. Effects on humans of solvents in the inspiratory air: A method for estimation of uptake. Environ. Res. 15:1-4.

Aviado, A. M., S. Zakhari, J. A. Simaan, and A. G. Ulsamer. 1976. Methyl chloroform and trichloroethylene in the environment. CRC Press. Cleveland, Ohio.

Axelson, O., K. Anderson, C. Hogstedt, B. Holmberg, G. Molina, and A. de Verdier. 1978. A cohort study on trichloroethylene exposure and cancer mortality. J. Occup. Med. 20:194-196.

Baden, J.M., M. Kelley, R.I. Mayze, and V.F. Simmon. 1979. Mutagenicity of inhalation anesthetics: Trichloroethylene, divinyl ether, nitrous oxide and cyclopropane. Br. J. Anaesth. 51:417-421.

Banerjee, S., and B. L. Van Duuren. 1978. Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA in vitro. Cancer Res. 38:776-780.

Barrett, H.M., and J.H. Johnston. 1939. The fate of trichloroethylene in the organism. J. Biol. Chem. 127:756-770.

Barrett, H.M., J.G. Cunningham, and J.H. Johnson. 1939. A study of the fate in the organism of some hydrocarbons. J. Indus. Hyg. Toxicol. 21:479-490.

Bartsch, H., C. Malaveille, A. Barbin, and G. Planche. 1979. Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues. Arch. Toxicol. 44: 249-277.

Bartonicek, V. 1962. Metabolism and excretion of trichloroethylene after inhalation by human subjects. Br. J. Ind. Med. 19:134-141.

Bell, Z.G., K.J. Olson, and T.J. Benya. 1978. Final report of audit findings of the Manufacturing Chemists Association (MCA): Administered trichloroethylene (TCE) chronic inhalation study at Industrial Bio-Test Laboratories, Inc., Decatur, Illinois. Unpublished.

Bolt, H.M., A. Buchter, L. Wolowski, D.L. Gil, and W. Bolt. 1977. Inculation of ^{14}C-trichloroethylene vapor with rat liver microsomes: Uptake of radioactivity and covalent binding of metabolites. Inter. Arch. Occup. Environ. Health 39:103-111.

Bonse, G., T. Urban, D. Reichert, and D. Henschler. 1975. Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem. Pharmacol. 24:1829-1834.

Bonse, G., and D. Henschler. 1976. Chemical reactivity, biotransformation, and toxicity of polychlorinated aliphatic compounds. CRC Crit. Rev. Toxicol. 4:359-409.

Bronzetti, G., E. Zeiger, and D. Frezza. 1978. Genetic activity of trichloroethylene in yeast. J. Environ. Pathol. and Toxicol. 1:411-418.

Butler, T. C. 1949. Metabolic transformation of trichloroethylene. J. Pharmacol. Exptl. Therap. 97:84-92.

Callen, D.F., C.R. Wolf, and R.M. Philpot. 1980. Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae. Mutation Research 77: 55-63.

Carcinogen Assessment Group. 1980. Method for determining the unit risk estimate for air pollutants. U. S. Environmental Protection Agency. 19 pp. Unpublished.

Cole, W.J., R.G. Mitchell, and R.F. Salamonsen. 1975. Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography. J. Pharm. Pharmacol. 27:167-171.

Cornish, H.H. 1975. Solvents and vapors. Pages 508-509 in Toxicology. L.J. Casarett and J. Doull, eds. The basic science of poisons. MacMillan, New York.

Daniel, J.W. 1963. The metabolism of Cl^{36}-labeled trichloroethylene and tetrachloroethylene in the rat. Biochem. Pharmacol. 12:795-802.

Demopoulos, H.B., B. Wagner, and J. Cimino. 1980. An academic review of the hazards posed by trichloroethylene. Unpublished document presented at EPA Science Advisory Board meeting on Air Carcinogens, Washington, D.C., Sept. 3-4, 1980.

Fahrig, R. 1977. The mammalian spot test (Fellfleckentest) with mice. Arch. Toxicol. 38:87-98.

Fahrig, R. 1978. Uber die Brauchbarkeit Von Mutagenitatstests als Krebs-vortest Vergleichende Untersuchungen im "Host-Mediated Assay" und "Sauger-Spot Test." Staub-Reinhalt. Luft 38(6):232-235.

Fernandez, J.G., B.E. Humbert, P.O. Droz, and J.R. Caperos. 1975. Exposition au trichloroethylene, Bilan de l'absorption, de l'excretion et du metabolisme sur des sujets humains. Arch. Mal. Prog. Med. Trav. Secun. Soc. 36(7-8):397-407.

Fishbein, L. 1979. Potential halogenated industrial carcinogenic and mutagenic chemicals. I. Halogenated unsaturated hydrocarbon. Sci. Total Environ. 11:111-161.

Forssman, S., and C. E. Holmquist. 1953. The relation between inhaled and exhaled trichloroethylene and trichloroacetic acid excreted in the urine of rats exposed to trichloroethylene. Acta. Pharmacol. Toxicol. 9:235-244.

Frankel, H.H., R.S. Yamamoto, E.K. Weisburger, and J.H. Weisburger. 1970. Chronic toxicity of azathioprine and the effect of this immunosuppressant on liver tumor induction by the carcinogen N-hydroxy-N-2-fluorenyl acetamide. Toxicol. Appl. Pharmacol. 17:462-480.

Greim, H., G. Bonse, Z. Radwan, D. Reichert, and D. Henschler. 1975. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochem. Pharmacol. 24:2012-2017.

Hathway, D. E. 1980. Consideration of the evidence for mechanisms of 1,1,2-trichloroethylene metabolism, including new identification of its dichloroacetic acid and trichloroacetic acid metabolites in mice. Cancer Letter 8:263-269.

Helliwell, P.J., and A.M. Hutton. 1950. Trichloroethylene anesthesia. I. Distribution in the fetal and maternal circulation of pregnant sheep and goats. Anesthesiology 5:4-15.

Henschler, D., and G. Bonse. 1977. Metabolic activation of chlorinated ethylenes. Dependence of mutagenic effect on electrophilic reactivity of the metabolically formed epoxides. Arch. Toxicol. 39:7-12.

Henschler, D., and G. Bonse. 1978. Metabolic activation of chlorinated ethylene derivatives. In: 20th Congress of the European Society of Toxicology (Abstracts). West Berlin pp. 1-8.

Henschler, D., E. Eder, T. Neudecker, and M. Metzler. 1977. Carcinogenicity of trichloroethylene: Fact or artifact? Arch. Toxicol. 37:233-236.

Henschler, D., W. Romen, H.M. Elasser, D. Reichert, E. Eder, and Z. Radwan. 1980. Carcinogenicity study of trichloroethylene by long-term inhalation in the animal species. Arch. Toxicol. 43:237-248.

Ikeda, M. 1977. Metabolism of trichloroethylene and tetrachloroethylene in human subjects. Environ. Health Perspec. 21:239-245.

International Agency for Research on Cancer (IARC). 1976. Trichloroethylene. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. 11:263-276.

Kimmerle, G. and A. Eben. 1973a. Metabolism, excretion and toxicology of trichloroethylene after inhalation. 2. Experimental human exposure. Arch. Toxicol. 30:127-138.

Kimmerle, G. and A. Eben. 1973b. Metabolism, excretion and toxicology of trichloroethylene after inhalation. 1. Experimental exposure on rats. Arch. Toxicol. 30:115-126.

Kirby, K.S. 1957. A new method for the isolation of deoxyribonucleic acids; Evidence on the nature of bonds between deoxyribonucleic acid and protein. Biochem. J. 66:495-504.

Klaasen, E.D., and G.L. Plaa. 1966. Relative effects of various chlorinated hydrocarbons on liver and kidney functions in mice. Toxicol. Appl. Pharmacol. 10:139-151.

Klaasen, C.D., and G.L. Plaa. 1967. Relative effects of various chlorinated hydrocarbons on liver and kidney functions in dogs. Toxicol. Appl. Pharmacol. 10:119-131.

Kleinfeld, M., and I.R. Tabershaw. 1954. Trichloroethylene toxicity. Indus. Hyg. Occup. Med. 10:134-141.

Konietzko, H.W., W. Haberlandt, H. Heilbronner, G. Reill, and H. Weichardt. 1978. Cytogenetische Untersuchungen an Trichlorathylen-Arbeitern. Arch. Toxicol. 40:201--206.

Kroes, R., J.M. Berkvens, and J.H. Weisburger. 1975. Immunosuppression in primary liver and colon tumor induction with N-hydroxy-N2-fluorenylacetamide and azoxymethane. Cancer Res. 35:2651-2656.

Krueger, G.R.F. 1972. Chronic immunosuppression and lymphomagenesis in man and mice. Natl. Cancer Inst. Monogr. 35:183-190.

Laham, S. 1970. Studies on placental transfer. Indus. Med. Surg. 39:46-49.

Laib, R.J., G. Stockle, and H.M. Bolt. 1978. Induction of pre-malignant hepatic lesions: Comparative effects of vinyl chloride and trichloroethylene. 20th Congress of the European Society of Toxicology. West Berlin. Abstract 67.

Laib, R.J., G. Stockle, H.M. Bolt., and W. Kung. 1979. Vinyl chloride and trichloroethylene: Comparison of alkylating effects of metabolites and induction of preneoplastic enzyme deficiencies in rats liver. J. Can. Res. Clin. Oncol. 94:139-147.

Land, P.E., E.L. Owen, and H.W. Linde. 1979. Mouse sperm morphology following exposure to anesthetics during early spermatogenesis. Anesthesiology 51(3): S 259.

Laskin, S., A.R. Sellakumar, M. Kuschner, S. Le Mendola, N. Nelson, G.M. Rusch, G.V. Katz, N.C. Dulak, and R.E. Albert. 1980. Inhalation carcinogenicity of epichlorohydrin in noninbred Sprague-Dawley rats. J. Natl. Cancer Inst. 65:751-755.

Lillian, D., H. Singh, A. Appleby, L. Lobban, R. Arnts, R. Gumpert, R. Hague, J. Toomey, J. Kazazis, M. Antell, D. Hansen, and B. Scott. 1975. Atmospheric fates of halogenated hydrocarbons. Environ. Sci. Technol. 9:1042-1048.

Loprieno, N., R. Barale, A.M. Rossi, S. Fumerjo, G. Meriggi, A. Mondino, and S. Silvestri. 1979. In vivo mutagenicity studies with trichloroethylene and other solvents (preliminary results). Unpublished.

Luz, A. 1977. The range of incidence of spontaneous neoplastic and nonneoplastic lesions of the laboratory mouse. Z. Versuchstierkd 19:342-343.

Maltoni, C. 1979. Results of long-term carcinogenicity bioassays of trichloroethylene experiments by oral administration on Sprague-Dawley rats. In press.

Margard, W. 1978. Summary report on in vivo bioassay of chlorinated hydrocarbon solvents. Unpublished.

Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Molec. Biol. 3:208-218.

Mazza, V., and A. Brancaccio. 1967. Comportamento degli elementi figurati del sangue a del midollo nella intossicazione sperimentale da tri. Folia. Med. 50:318-324.

McConnell, G., D.M. Ferguson, and C.R. Pearson. 1975. Chlorinated hydrocarbons and the environment. Endeavor 34:13-18.

Mondino, A. 1979. Examination of the live mutant activity of the trichloroethylene compound with "Schizosaccharomyces pombe." Unpublished.

Monster, A. C. 1979. Difference in uptake, elimination, and metabolism in exposure to trichloroethylene, 1,1,1-trichloroethane and tetrachloroethylene in volunteers, influence of workload and exposure concentration. Inter. Arch. Occup. Environ. Health 42:311-317.

Monster, A.C., G. Boersma, and W.C. Duba. 1976. Pharmacokinetics of trichloroethylene volunteers. Inter. Arch. Occup. Environ. Health 42:87-102.

Monster, A.C., G. Boersma, and W.C. Duba. 1979. Kinetics of trichloroethylene in repeated exposures of volunteers. Inter. Arch. Occup. Environ. Health 42:283-292.

Monsinger, M., and H. Fiorentini. 1955. Reaction hepatque, renales, ganglionnaires et spleniques dans l' intoxication experimentale par le trichloroethylene chez le chat. C. R. Soc. Biol. (Paris) 149:150-172.

National Cancer Institute (NCI). 1976. Carcinogenesis bioassay of trichloroethylene. CAS No.79-01-6. NCI-CG-TR-2.

National Institute for Occupational Safety and Health (NIOSH). 1973. Criteria for a recommended standard: Occupational exposure to trichloroethylene. HEW Publ. HSM 73-11025.

Nomiyama, K., and H. Nomiyama. 1971. Metabolism of trichloroethylene in humans. Inter. Arch. Arbeitsmed. 28:37-48.

Nomiyama, K., and H. Nomiyama. 1974. Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Inter. Arch. Arbeitsmed. 32:75-83.

Ogli, K. 1977. Reversible growth suppressing effects of various anesthetics in *Euglena gracilis*. Med. Jour.of Osaka Univer. 28(1):15-21.

Okuda, C., and K. Ogli. 1976. The effects of anesthetics on microtubule protein. Jap. J. Anesthesiology 25:785-791.

Prendergast, J.A. , R.A. Jones, L.J. Jenkins Jr., and J. Siegel. 1967. Effects on experimental animals on long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl. Pharmacol. 10:270-289.

Price, P.J., C.M. Hassett, and J.I. Mansfield. 1978. Transforming activities of trichloroethylene proposed and industrial alternatives. *In Vitro* 14:290-293.

Purchase, I.F.H., E. Longstaff, J. Ashby, J.A. Styles, D. and P.A. Lefevre, and F.R. Westwood. 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens and recommendations for their use. Brit. J. Cancer 37:873-959.

Rudali, G. 1967. Potential carcinogenic hazards from drugs. Evaluation of risks. U.I.C.C. Monograph Series 7:138-143.

Russell, L.B., and B.E. Matter. 1980. Whole-mammal mutagenicity tests. Evaluation of five methods. Mutation Research 75: 279-302.

Sanders, V.M, A.N. Tucker, P.H. Hallett, B. Kaufman, R.A. Carchman, and A.E. Munson. 1980. Immunotoxicology of trichloroethylene in the mouse. II. Effects on humoral and cell-mediated immunity. In press.

Sato, A., and T. Nakajima. 1978. Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. Brit. J. Indus. Med. 35:43-49.

Schairer, L.A., J. Van't Hof, C.G. Hayes, R.M. Burton, and F.J. deSerres. 1978. Measurement of biological activity of ambient air mixtures using a mobile laboratory for in situ exposures. Preliminary results from the Tradescantia plant test system. EPA Pub. 600/9--78--027:421-440.

Seifter, J. 1944. Liver injury in dogs exposed to trichloroethylene. J. Indus. Hyg. Toxicol. 26:250-253.

Shahin, M.M., and R.C. Von Borstel. 1977. Mutagenic and lethal effects of α-benzene hexachloride, dibutyl phthalate and trichloroethylene in Saccharomyces cerevisiae. Mutat. Res. 48:173-180.

Siegel, J., R.A. Jones, R.A. Coon, and J.P. Lyon. 1971. Effects on experimental animals of acute, repeated and continuous inhalation exposures to dichloroacetylene mixtures. Toxicol. Appl. Pharmacol. 18:168-174.

Simmon, V.F., K. Kauhanon, and R.G. Tardiff. 1977. Mutagenic activity of chemicals identified in drinking water. Pages 249-258 in D. Scott, B. A. Bridges, and F. H. Solves, eds. Progress in genetic toxicology. Elsevier/North Holland Biomedical Press.

Slacik-Erben, R., R. Roll, G. Franke, and H. Uehleke. 1980. Trichloroethylene vapours do not produce dominant lethal mutations in male mice. Arch. Toxicol. 45: 37-44.

Smyth, H.F., Jr., C.P. Carpenter, C.S. Weil, U.C. Pozzani, J.A. Striegel, and J.S. Nycum. 1969. Range-finding toxicity data: List VII. J. Amer. Indust. Hyg. Assoc. 30:470-476.

Soucek, B., and D. Vlachova. 1960. Excretion of trichloroethylene metabolites in human urine. Brit. J. Indus. Med. 17:60-64.

Soucek, B., J. Tiesinger, and E. Pavelkova. 1952. The absorption and elimination of trichloroethylene in man. Pracov. Lek. 4:31-41.

Stewart, R.D., and H.C. Dodd. 1964. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through human skin. Amer. Indust. Hyg. Assoc. J. 25:439-446.

Stewart, R., H. Dodd, H. Gay, and D. Erley. 1970. Experimental human exposure trichloroethylene. Arch. Environ. Health 20:64.

Stott, W.T., J.F. Quast, and P.G. Watanabe. 1981. The pharmacokinetics and molecular interactions of trichloroethylene in mice and rats as related to oncogenicity. Final report to Chemical Manufacturers Association. Ref. No. TCE 2.0-PHKS/MACM. 33 pp.

Sturrock, J.E., and J.F. Nunn. 1975. Mitosis in mammalian cells during exposure to anesthetics. Anesthesiology 43(1):21-33.

Styles, J.A. 1977. A method for detecting carcinogenic organic chemicals using mammalian cells in culture. Brit. J. Cancer 36:558-563.

Styles, J.A. 1979. Cell transformation assays. Pages 147-164 in G.E. Pagett, ed. Topics in Toxicology: Mutagenesis in Sub-mammalian Systems: Status and Significance. University Park Press, Baltimore, MD.

Tola, S., R. Vilhuner, E. Jaruinen, and M.L. Korkale. 1980. A cohort study on workers exposed to trichloroethylene. J. Occup. Med. 22:737-740.

Tucker, A.N.M., V.M. Sanders, D.W. Barnes, T.J. Bradshaw, and A.E. Munson. 1980. Immunotoxicology of trichloroethylene in the mouse. I. General toxicology. Unpublished.

Uehleke, H., S. Poplawski-Tabarelli, G. Bonse, and D. Henschler. 1977. Spectral evidence for 2,2,3-trichloro-oxirane formation during microsomal trichloroethylene oxidant. Arch. Toxicol. 37:95-105.

Uehleke, H., and S. Poplawski-Tabarelli. 1977. Irreversible binding of ^{14}C-labelled trichloroethylene to mice liver constituents *in vivo* and *in vitro*. Arch. Toxicol. 37:289-294.

U. S. Environmental Protection Agency (EPA). 1976. Interim procedures and guidelines for health risk and economic impact assessments of suspected carcinogens. Federal Register 41:21402 (May 25).

U. S. Environmental Protection Agency (EPA). 1979. Ambient air carcinogenic vapors. Improved sampling and analytical techniques and field studies. EPA-600/2-79-081.

U. S. Environmental Protection Agency (EPA). 1980. Environmental sources of trichloroethylene exposure. Source contribution factors. EPA-56/11-80-009.

Van Duuren, B.L., L. Langseth, B.M. Goldschmidt, and L. Orris. 1967. Carcinogenicity of epoxides, lactones, and peroxy compounds. VI. Structure and carcinogenic activity. J. Natl. Cancer Inst. 39:1217-1228.

Van Duuren, B.L., and S. Banerjee. 1976. Covalent interaction of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res. 36:2419-2422.

Van Duuren, B.L., B.M. Goldschmidt, G. Lowengart, A.C. Smith, S. Melchionne, I. Seldman, and D. Roth. 1979. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. J. Natl. Cancer Inst. 63:1433-1439.

Waskell, L. 1978. A study of the mutagenicity of anesthetics and their metabolites. Mutat. Res. 57:141-153.

Waters, E.M., H.B. Geistner, and J.E. Huff. 1977. Trichloroethylene. I. An overview. J. Toxicol. Environ. Health 2:671-707.

White, A.S., S. Takehisa, E.I. Eger II, S. Wolff, and W.C. Stevens. 1979. Sister-chromatid exchanges induced by inhaled anesthetics. Anesthesiology 50: 426-430.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1982 -559-014/0818